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THE UNIVERSITY OF ALBERTA

CULTIVATION AND DEHYDRATION OF OYSTER MUSHROOMS

by

© BRIGITTE LI-SHING-TAT

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF FOOD SCIENCE

EDMONTON, ALBERTA
SPRING, 1988

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...A.Y.B. Li Shing Tat.........

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled: CULTIVATION AND DEHYDRATION OF OYSTER MUSHROOMS submitted by BRIGITTE LI-SHING-TAT in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE IN FOOD SCIENCE.

Supervisor

Date: Feb 11/1988

Dedicated to
my Parents

ABSTRACT

The objectives of this study were: (a) to evaluate the use of Aspen shavings for the cultivation of Oyster mushrooms (*Pleurotus sajor-caju* and *Pleurotus ostreatus*) and (b) to investigate some alternative processing methods for drying *Pleurotus sajor-caju* mushrooms. Aspen wood per se produced a very low yield of mushrooms (3% fresh mushrooms/100 g wet substrate, from the first flush). Soaking the shavings in liquid cottage cheese whey increased the yield more than two-fold but was still inadequate for commercial application. The whey-soaked substrate was also particularly susceptible to infection by the green mold, *Trichoderma*. Supplementation with 0%, 3%, or 5% of canola meal (g/100g wet substrate) gave a yield of 4.9%, 16.6%, and 22.1% (from two flushes) respectively. At a higher level of supplementation (5% and 7%), mycelial growth was inhibited. Fruitbodies harvested from supplemented substrates had a higher nitrogen content than those from the Aspen shavings only. A normal (P_2) and a sporeless (Sp_2) strain of *Pleurotus ostreatus* were included in the cultivation experiments. The Sp_2 fruitbody had a very large stem to cap ratio, making it undesirable for consumer acceptability since the stem is tougher in texture. Scanning electron microscopy of the gill surface of the Sp_2 strain showed that there were no spores present.

The caps of *Pleurotus sajor-caju* mushrooms were found to lend themselves well to air-, freeze-, or high-vacuum drying. The quality of the freeze- and vacuum-dried products was comparable to each other. They both retained the shape and colour close to that of the fresh mushroom, rehydrated within two minutes to a similar rehydration capacity and had a similar texture. In contrast, the air-dried mushrooms were shrunken and darker, took forty minutes to rehydrate, and were tougher in texture. Scanning electron microscopy showed less damage to the gill surface of the vacuum-dried product compared to the freeze-dried sample, but the

hyphae of both samples looked similar in that they had maintained their tubular structure to a certain extent. The basidia of the air-dried samples showed considerable collapse while the hyphae were flattened. Sensory evaluation indicated no significant difference in the flavour and overall acceptability of the vacuum- and freeze-dried mushrooms but the vacuum-dried samples were significantly better than the air-dried samples. Since there is no freezing stage involved in the high-vacuum drying method, any microstructural damage due to the freezing process would be avoided. High-vacuum drying method is of potential importance for drying mushrooms, especially for those commanding high prices such as the Chanterelles.

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CHAPTER 1

INTRODUCTION

1.1 EDIBLE MUSHROOMS OF COMMERCIAL IMPORTANCE

As food items, mushrooms are highly prized for their flavour. Although to many North American consumers, the most familiar edible mushroom is the button mushroom, there are more than 2,000 edible species [Chang and Miles, 1982], each with its unique flavour. While there are many varieties of wild mushrooms available on the market of various European countries [Spencer, 1985], the number of commercially cultivated species is, however, limited to only a few. The five most important ones are the button mushroom (*Agaricus bisporus*), the black forest mushroom (*Lentinus edodes*), the winter mushroom (*Flammulina velutipes*), the straw mushroom (*Volvariella volvacea*), and the Oyster mushrooms (*Pleurotus* spp.) [Chang and Miles, 1982]. It is estimated that in 1983-1984 the annual worldwide production of edible cultivated mushrooms was about one and a half million tonnes, 70% of which were contributed by *Agaricus bisporus* [Chang and Miles, 1987]. The production for 1987 is projected to be two million tonnes [Jelen, personal communication, 12th Mushroom Congress, 1987].

1.1.1 *Agaricus bisporus*

The history of mushroom cultivation started with the button mushroom or Champignon de Paris. Melon growers in the vicinity of Paris are reported to have initiated the cultivation in 1650 on spent compost used for production of melons [Royse and Schisler, 1980]. This mushroom is now grown in many countries, the major producers being USA, France, China, Taiwan, and Holland [Courvoisier, 1984]. Several steps are involved in the cultivation of the button mushroom: compost preparation, spawning and spawn-running, casing, and cropping. For composting, the substrate (usually wheat straw and horse manure) is wetted, mixed with some ingredients (gypsum, phosphate and a nitrogen source), and stacked into piles. The stacks are turned at intervals of two to three days for a period of 7 to 14 days. During the process, the temperature inside the stacks can reach from 70°C to 80°C. The composted substrate is then packed into trays or shelves and pasteurised at 50°C to 60°C. After cooling, the compost is inoculated with spawn and then incubated for 2-4 weeks at 20°C to 24°C in dark, humid (95% R.H) conditions. To induce the formation of fruitbodies, the air is kept fresh by ventilation and the temperature is reduced to 15°C, the humidity of the atmosphere is set at 90% and the compost is covered with a casing layer of soil (often a mixture of sphagnum peat and chalk). The time taken from the application of the casing layer to the harvest of the first mushrooms ranges from 18 to 21 days. The mushrooms are produced in a succession of batches called flushes. Each flush of mushrooms is harvested over a period of about 4 days, and flushes occur at intervals of 7 to 10 days [Gaze, 1985; Oddson, 1979]. *Agaricus bisporus* and the various strains of this species are the mushrooms most commonly consumed in Canada [Patrick *et al.*, 1983].

1.1.2 *Lentinus edodes*

The black forest mushroom or Shiitake (*L. edodes*) is the most important cultivated mushroom in Japan. Traditionally, Shiitake has been grown on Oak (*Quercus serata*, *Q. acuticima* etc.) tree logs . The logs are cut about 3 metre long and 5-15 cm in diameter, drilled with holes, and inoculated with a wood plug spawn inserted into each hole, which is then covered with wax. The logs are then laid side by side at a slant and covered. After about one year, the mycelium will have permeated throughout the wood and the logs are raised into a more upright position to favour fruiting. Fruitbodies first appear in two years and production will continue for up to six or seven years [Farr, 1983]. Recently innovations have been made into Shiitake cultivation using sawdust or wood chips with various additives under controlled conditions. One method described by Patrick *et al.*, [1983] is as follows. Substrate is packed into a container (e.g plastic bags), sterilised, inoculated with spawn and incubated for 15 weeks. For induction, the artificial 'logs' are submerged in cold water (15-16°C) for 24 hours, then given another cold treatment at 10-15°C; light (400 lux of 400-700 nm) is provided. Mushrooms appear after 10-12 days and can be collected from 2 flushes . Apart from Japan, other important producers of Shiitake are China, Korea, and Taiwan. Recently Singapore, New Zealand and Thailand have initiated production while many other countries are still experimenting with Shiitake farming [Miles and Chang, 1985]. The total world production of Shiitake was 234,000 tonnes in 1983-1984 [Chang and Miles, 1987].

1.1.3 *Flammulina velutipes*

The Winter mushroom or Enokitake (*F. velutipes*) is a small mushroom commercially cultivated in Japan and Taiwan. Enokitake mushrooms are grown on a mixture of sawdust and rice bran (4:1) filled into polypropylene bottles (800-1000 mL). The substrate is sterilised, inoculated, and incubated between 20-25°C in the dark with the bottles capped. Twenty to twenty-five days after inoculation, the caps are removed from the bottles, the temperature is lowered to 10-12°C, and the relative humidity of the air set at 80-85%. Primordia appear after 10-14 days. For the normal development of the fruitbodies, light is provided and the temperature is lowered to 3-5°C. When the stumps have grown to 2 cm high, the temperature is held at 5-8°C and the neck of the bottle is wrapped with waxed paper or a plastic film to hold the fruitbodies upright until they are mature and harvested [Tonomura, 1978; Zadrazil and Grabbe, 1981; Farr, 1983]. The production of Enokitake in Japan was reported to be 63,000 tonnes in 1984 [Mori, 1987].

1.1.4 *Volvariella volvacea*

The Straw mushroom (*V. volvacea*) is grown in the tropical and sub-tropical regions of Asia and Africa because of its high temperature requirements [Santiago, 1983]. Initially paddy rice straw was used for cultivation but other substrates such as water hyacinth, oil palm bunch, banana leaves, or cotton waste have been found to be suitable [Chang, 1983]. The most primitive technique consists of stacking the straw in layers or bundles, moistening it with water, followed by inoculation, and incubation either outdoors or in enclosed spaces. The optimum temperature for incubation is 34-35°C. About 10 days after inoculation, primordia appear and the first mushrooms are ready to be picked after a further 3-4 day period [Farr, 1983]. More sophisticated techniques involving fermentation and pasteurisation of the substrate and the use of carefully controlled conditions for the formation of the fruitbodies are described by Chang [1982]. Fruitbodies are usually marketed at the button stage, when the cap is still enclosed by the volva or at the "egg" stage, just after the volva has been ruptured. About 65,000 tonnes were produced in 1983-1984 [Chang and Miles, 1987], the major producers being China, Taiwan, Thailand, and Indonesia [Chang, 1983].

1.1.5 *Pleurotus* spp.

The cultivation of the various *Pleurotus* spp. (Oyster) mushrooms is a relatively small and new industry. Royse and Schisler [1980] reported that of the total world production of mushrooms, the Oyster mushroom contributed only 1.3% compared to a value of 73.2% and 14.2% for the button mushroom and Shiitake respectively. By 1983, this figure rose to 3% with an estimated 35,000 tons [Hayes, 1985]. The growing interest in the Oyster mushroom can be attributed to several factors. The mushroom has a good texture and flavour, it is relatively easy to grow and it will thrive on a variety of cellulosic materials. Also, there is a wide range of edible species which are adapted to different climates, from tropical to temperate conditions [Jablonski, 1984]. Details of the cultivation steps are given in Chapter 2.

1.1.6 Other species

Other mushroom species that are commercially cultivated include *Auricularia* spp. (the Wood Ear fungus), *Pholiota Nameko*, *Tremella fuciformis* (the White jelly fungus or the Silver Ear), *Dictyophora duplicata*, *Stropharia rugoso-annulata* [Chang and Miles, 1987; Chang, 1982; Santiago, 1983]. In adddtion to the cultivated ones, some mushrooms (e.g Chanterelles, Morels, Boletes, Truffles) are collected wild, on a scale large enough to support small businesses. Although already a tradition in many European countries, the picking of wild mushrooms for commercial purposes is rapidly gaining popularity in North America, but concerns have been voiced about the safety of this practice. The U.S. Food and Drug Administration issued a recommendation that "wild mushrooms are not to be sold or served through a retail establishment unless they have been individually inspected and determined to be safe by a specialist acceptable to the regulatory authority" [Coombs and Rogers, 1987].

1.2 NUTRITIONAL VALUE OF MUSHROOMS

Since mushrooms belong to a wide range of species, they show great variation in their composition and therefore in their nutritional value. However, in terms of nutrient content, some edible mushrooms fare well, if not better, when compared to most vegetables. Fresh mushrooms have a moisture content ranging from 85-90% and a caloric value of 20-30 kcal/100 g. They contain varying amounts of protein, carbohydrate, fiber, fat, vitamins and minerals. Information for this review is summarised from the following references: Altamura *et al.* [1967]; Crisan and Sands [1978]; Delmas [1978]; Oddson [1979]; Chang [1980]; Guthrie [1983]; Manning [1985]; Li and Chang [1985]; Alexander [1987].

1.2.1 Protein

The protein content for various strains of *A. bisporus* ranges from 1.9-4.5%; for *Pleurotus*, 2.3-5%; and for *Volvariella*, 2.2-5% (fresh weight). These values, however, may not reflect the actual protein content of the mushroom. Usually the protein content of foods is estimated by multiplying the total nitrogen content by a factor of 6.25, following the assumption that most proteins contain 16% nitrogen and that they are almost 100% digestible. Numerous feeding studies using rats and human subjects have shown that the digestibility of mushroom proteins varied from 71-90%. A conversion factor of 4.38 (70% x 6.25) was therefore recommended. This reduced digestibility can be partly explained by the fact that fungi contain a significant amount of nitrogen as chitin in their cell wall. Chitin is a polymer of N-acetyl-D-glucosamine units and is not digestible. In addition, mushrooms contain a significant amount of free amino acids such as glutamic acid, proline, alanine, aspartic acid, lysine, ornithine, and serine. Several amines of physiological importance may also be present; they include taurine, sarcosine, and creatinine.

In general, the protein content of some fresh mushrooms is about twice that of vegetables such as asparagus and cabbage but is lower than that of green peas, Brussels sprouts, and pulses (beans, dried peas, lentils). The protein value of any food depends not only on the quantity but also on its amino acid composition. *Agaricus bisporus*, *Volvariella volvacea*, and *Pleurotus* spp. contain all the essential amino acids but in different proportions. Thus the lysine average for *Agaricus* is almost the same as that of *Volvariella* but is almost twice that of *Pleurotus*. Among the *Pleurotus* species, *P. sajor-caju* has a higher concentration of the sulphur containing amino acids than *P. ostreatus*.

The use of mushrooms as a protein source for human consumption has been discussed by several authors [Flegg and Maw, 1976; Delcaire, 1981; Sohi, 1982]. However, given the fact that mushrooms are about 90% water and 2-4% protein, and that a normal serving would be 100-200 g, it is unlikely that they could contribute a significant amount to the protein needs of an adult.

1.2.2 Carbohydrate and Fiber

Carbohydrates are usually the main constituent of mushrooms after water. The carbohydrate content of fresh mushrooms varies from 5-6 % for the button mushroom, 4-8% for the Oyster mushroom, and 3-9% in the Shiitake mushroom. The carbohydrates include mono- and disaccharides, glycogen, mannitol, uronic acids and amino sugars, cellulosic compounds and glucans. The simple sugars are mostly glucose, galactose, xylose, rhamnose, mannose and, arabinose. The most common disaccharide is trehalose. The major constituent of fiber is chitin, a structural component of the cell wall. In the button mushroom, fiber accounts for 0.5-0.6% of the fresh weight of the fruitbody.

1.2.3 Fat

The fat content of most mushrooms is usually quite low ranging from 0.2-0.8% fresh weight. In *Agaricus bisporus*, phospholipids constitute 75% of the fat. Of the fatty acids present, linoleic acid makes up 75% of the total, followed by palmitic acid (15%), and stearic acid (5%). Mono-, di-, and triglycerides, sterols, and sterol esters are also present. Sphingolipids, an important group of lipids present in the brain and the central nervous system, have also been found in very small quantities in mushrooms.

1.2.4 Vitamins and minerals

Button mushrooms are fairly good sources of the B vitamins, particularly thiamine, niacin, and riboflavin. Table 1 compares the value of some vitamins for *A. bisporus* with those of lettuce and cabbage.

The average recommended daily intake for an American adult are 1-1.5 mg of thiamine, 1.2-1.7mg riboflavin, and 13-19 mg niacin. A 100 g portion of the button mushrooms would therefore supply 25-37% and 27-40% of the riboflavin and niacin required respectively but less than 10% of the thiamine. Analysis of the button mushrooms showed that they contain 0.32-0.65 µg of vitamin B_{12} per 100 g portion. Since the daily requirement for this vitamin in an adult is 3 µg, a 10 g portion would provide enough of B_{12}. The vitamin C content of *P. ostreatus*, *P. sajor-caju*, *V. volvacea*, and *L. edodes* is very low ranging from 0.66-5.07 mg per 100 g (fresh weight basis). Since the daily requirement of vitamin C for an adult is 60 mg, a 100 g portion of mushrooms would contribute very little. Fat soluble vitamins A, D, E are present in trace amounts because of the low lipid content in mushrooms.

The minerals present in the button mushroom in any significant amount include phosphorus, sodium, and potassium. Calcium and iron are present in relatively small amounts. The mineral content ranges from 8-11% on a dry weight basis.

Table 1 Average values of some B vitamins in mushrooms (*A. bisporus*), lettuce, and cabbage (mg/100g fresh weight)

Vitamin	*A. bisporus*	Lettuce	Cabbage
Thiamine	0.1	0.06	0.05
Riboflavin	0.44	0.08	0.04
Niacin	5.20	0.32	0.32

Data from Souci *et al.*, [1981]

1.3 MEDICINAL VALUE

Medicinal properties have been attributed to mushrooms in the foklore medicine of several cultures [Cochran, 1978]. Recently, some of the reported medicinal properties have been studied and interesting observations have been made concerning some mushrooms that are already being consumed.

1.3.1 Hypocholesterolemic effect

Kaneda *et al.*, [1966] fed rats with 5% ground Shiitake for ten weeks and observed a decrease in the average plasma cholesterol by 22%. These authors also found that if the rats were given a diet containing 1% cholesterol, supplementation of the diet with 5% of some dried mushrooms (*Lentinus edodes*, *Agaricus bisporus*, *Auricularia polytrichia*, *Flammulina velutipes*) reduced the plasma cholesterol . *Auricularia polytrichia* or *Flammulina velutipes* were less effective than *Lentinus edodes* and *Agaricus bisporus*. Yamamura and Cochran [1976] fed mice with dried Shiitake for a more extended period of 14 months and reported that the hypocholesterolemic effect was maintained throughout. Saitoh [1976], however, failed to show any effect in rabbits. Suzuki and Ohshima [1976] carried out some experiments to determine whether there was any effect in humans. Three groups of 10 women were fed fresh, dried, and irradiated dry Shiitake and showed a decrease in their serum cholesterol by 12%, 7%, and 6% respectively. In addition, if Shiitake was eaten with butter, a decrease of 4% in serum cholesterol was observed while there was a 14% increase in the control. The hypocholesterolemic effect of Shiitake was attributed to a compound called Eritadenine. This substance was found to lower the levels of serum cholesterol, triglycerides, and phospholipids in rats [Takashima *et al.*, 1973] but Saitoh [1976] could not demonstrate the hypocholesterolemic effect in humans. Tokuda and Kaneda [1979] attributed the hypocholesterolemic effect of Eritadenine to an acceleration of cholesterol metabolism which induces an increase in cholesterol excretion.

1.3.2 Antitumour effects

According to Ames [1983], the human diet contains a great variety of natural carcinogens as well as many anticarcinogens. Foods and compounds believed to have anticarcinogenic activity are the cruciferous vegetables (such as broccoli, cabbage, Brussels sprouts), vitamin C, vitamin E, and beta-carotene [Rensberger, 1984]. Evidence is now accumulating that some edible mushrooms may also contain some anticarcinogenic or antitumour substances. Hamuro *et al.* [1976] prepared hot water extracts of *Agaricus bisporus* and *Lentinus edodes*. The extracts were sterilised and injected intraperitoneally into mice which had Sarcoma-180 transplanted under their skin. With the *Lentinus edodes* extract, there was an 80% tumor inhibition ratio and complete regression in 6 out of 10 mice, while for the *Agaricus bisporus* extract, the inhibition ratio was 12% and none of the mice showed any complete regression. Yoshioka *et al.* [1972] found that with *P. ostreatus*, there was a 75% tumour inhibition ratio and that the tumours in 5 of the 10 mice completely regressed. The substances responsible for the antitumour activities are believed to be polysaccharides [Chihara, 1979; Yoshioka *et al.*, 1975]. It is suggested that rather than acting directly on the cancer cells, these substances act as immunopotentiators, stimulating the body's intrinsic resistance against cancer [Hamuro *et al.*, 1976]. Mori [1987] demonstrated that the antitumour effects could also be observed when the mushrooms were taken orally. Mice with Sarcoma-180 were fed a diet containing 20% mushroom powder. Species tested were *Lentinus edodes*, *Grifiola frondosa*, *Agaricus bisporus*, *Pleurotus ostreatus*, *Flammulina velutipes*, *Pholiota glutinosa*, *Tremella fuciformis*, *Auricularia minor*, and *Volvariella volvacea*. All the species induced tumor inhibition ranging from 61% for *F. velutipes* to 86% for *G. frondosa*. Furthermore, Mori [1987] showed that various species differed in their ability to inhibit different types of carcinoma. Whether these results can be extrapolated to humans needs to be investigated. Clinical studies are being planned in Japan [Mori, 1986, personal communication]. If these results turn out to be positive, it is conceivable that mushrooms could be incorporated in the therapeutic diets for cancer patients as a part of the treatment. Since some anticancer substances may be toxic in large doses causing liver damage, dietary sources might be preferable [Rensberger, 1984].

1.3.3 Other effects

Other medicinal effects attributed to edible mushrooms include antibacterial, antifungal, antiprotozoal, and antiviral effects [Cochran, 1978]. Suzuki *et al.* [1976] found that the aqueous extract of *L.edodes* was effective against influenza A/SW-15 virus infection in mice. They suggested that the extract might stimulate interferon production. Hikino *et al.* [1985] observed significant hypoglycemic activity in mice injected with a water soluble extract of the mushroom, *Ganoderma lucidum*. They isolated the active hypoglycemic principles as two glycans, Ganoderans A and B.

1.4 BIOLOGICAL IMPORTANCE

Fungi play a vital role in the ecosystem as decomposers. Together with bacteria, they break down dead organic matter into its constituent compounds and elements which are thereby returned to the soil. Since many of the cultivated mushrooms are lignocellulolytic, they could be used to treat agricultural wastes while at the same time producing food. Chang [1982] estimated that more than 25% of the cereal straws annually produced are burnt both in the developed and underdeveloped countries. He calculated that if 25% of the straws were used to grow edible mushrooms, and assuming a biological efficiency of 50%, then each of the 4,182 million people in the world in 1978 could have been provided with a daily portion of 206 g of fresh mushrooms. In underdeveloped countries, emphasis should be on the cultivation of species that are best adapted to the local climate using the least costly methods of production. *Pleurotus* species are particularly promising in these respects since they can be grown on a wide variety of agricultural and industrial wastes (such as paddy straw, corn cobs, cotton waste, cotton seed hulls, and sawdust), and do not require strictly controlled growing conditions [Chang and Miles, 1984].

One of the factors limiting the use of wheat straw and wood for cattle feed is the presence of lignin, which is undigestible. It has been suggested that prior fermentation of the plant materials with lignocellulolytic mushroom species could be used not only to produce fruitbodies but also to improve the digestibility of the spent substrate. Zadrazil [1977] reported that the in vitro digestibility of wheat straw was increased from 40 to 57% after 120 days of fermentation with *P. ostreatus*. Sosulski [1985] found that *P. sajor-caju* degraded lignin less efficiently than *P. ostreatus*. The suitability of the spent substrate would eventually have to be tested in animal studies. Few in vivo digestibility studies using the spent mushroom compost have so far been reported. Bakashi *et al.* [1985] fed buffaloes with wheat straw previously used for *Pleurotus* spp. cultivation and found that the dry matter digestibility of the spent straw was lower than that of the raw straw. The organic matter in the raw straw was found to be lower due to the harvesting of the *Pleurotus* fruitbodies which had utilised 52% of the organic matter. Although the crude protein was increased from 2.49 to 4.7% of the dry matter, the higher intake of nitrogen was cancelled by an accompanying increase of feacal and urinary nitrogen. Calzada *et al.* [1987] found that the digestibility of the dry matter of spent substrate from *P. sajor-caju* was 55% while that of the raw straw was 52% when fed to lambs. These two studies showed that the spent substrate from which *Pleurotus* spp. have been grown and harvested did not bring any significant improvement in the feed value for the animals studied, but they indicated that the spent substrate was acceptable to these animals. Further animal studies would have to be carried out to evaluate the feed value of the spent substrate. So far, it seems that for the use of animal feed, a fermented substrate from which no fruitbodies have been harvested would be better.

Bisaria *et al.* [1983] have suggested generating biogas from the spent substrate. They found that spent rice straw used for *P. sajor-caju* cultivation enhanced the biogas yield by 54% compared to the raw straw. After fermentation, the residual solids from the digester could still be used as organic fertilisers. A mushroom farm could therefore integrate mushroom cultivation, biogas production for part of its energy requirements and the provision of the spent substrate as biofertiliser.

1.5 ECONOMIC IMPORTANCE

Mushroom cultivation is a multi-million dollar industry for the major producers of the world. The annual production of the button mushroom is estimated to be about 940 thousand tons and worth about US$ 14 billion while that of Shiitake totals about 192 thousand tons with an equivalent value of about US$

540 million [Chang and Miles, 1984]. The total domestic production of mushrooms in the United States in 1983/84 was about 246,689 tons and the sales value was US$ 471.4 million [USDA, 1984]. In Canada, the total production for 1985/86 was 49,706 tons with a farm value of Cd$ 124.259 million [Statistics Canada, 1987]. Compared to other vegetables, mushrooms fetch a relatively good price on the market. In Canada, mushrooms are the third most valuable vegetable crop after tomatoes and potatoes [Rinker, 1986]. In addition to the more commonly cultivated mushrooms, there are some mushroom species which command a much higher price. Included are those that are collected wild such as the Chanterelles (*Cantharellus cibarius*); Boletes (*Boletus edulis*); and Morels (*Morchella esculenta*). *Dictyophora duplicata*, considered a mushroom delicacy, sells for US$ 400-1,000 for 1 kg dried and can now be cultivated [Chang and Miles, 1984].

The United States, France, and the Federal Republic of Germany are reported to consume more than 50% of the world production of the button mushroom while Japan, Hong-kong, and China are the primary consumers of Shiitake mushrooms [Chang and Miles, 1984]. Canadians consume 5% of the world's production of mushrooms and the indication is that consumption is increasing, from 10.5 million kg in 1964 to 65.6 million kg in 1984 [Rinker, 1986]. According to Delcaire [1978], there is an increasing consumption of the button mushroom with a rise in the *per capita* 'private expenditure'. While this may have been true for many years in several countries where the button mushrooms are considered a luxury food item, there is now a changing attitude towards mushroom consumption. The improvement in cultivation techniques and the spread of mushroom production to underdeveloped and developing countries have brought down the market prices and tightened the competition in the mushroom business. China, Taiwan, and South Korea export a significant amount of canned mushrooms to the United States

[Owens *et al.*, 1986]. Fresh and processed mushrooms of the commonly cultivated species are now available at a reasonable cost to most consumers.

1.6 GENERAL OBJECTIVES OF THE INVESTIGATIONS

In order to sustain the growth in the mushroom industry, mushroom consumption should be encouraged and a greater variety of mushrooms should be explored for cultivation to offer a wider choice to the consumer. While in many Asian countries, several types of mushrooms have already been cultivated for many years, the focus in North America, Europe, and Australia has been on the button mushroom. Recently, however, some European countries have been actively involved in developing the Oyster mushroom industry and today, *Pleurotus* spp. are the second most important group of edible fungi grown in Europe next to *Agaricus bisporus* [Lelley, 1982]. In Canada, the *Pleurotus* mushroom industry has started only recently, mainly in British Columbia and Ontario. Compared to the knowledge available for *Agaricus bisporus*, relatively little research has been done on the cultivation and the processing of *Pleurotus* spp. mushrooms.

Aspen trees (*Populus tremuloides* Michx.) constitute a large proportion of the poplar population in Alberta. Making up 40% of the forest resources, the utilisation of poplar wood, however, amounts to only 1% of the total. Decay and stain are identified as the main factors limiting its use in the plywood and pulp mill industries. As a result there is an enormous amount of waste at the logging and mill sites. In addition, a large volume of by-products such as shavings, chips and sawdust are generated by the sawmills. Instead of going to waste, some of this biomass of poplar wood could be used to cultivate edible mushrooms. In nature, Oyster (*Pleurotus*) mushrooms are found on dead decaying aspen logs [Hiratsuka and Loman, 1984]. Several authors [Anderson, 1968; Vessey, 1969] have suggested using aspen logs and stumps for extensive cultivation of Oyster mushrooms. Lanzi

[1987] reported that in parts of Italy, small poplar logs are used to grow Oyster mushrooms for home consumption. One of the aims of this study was to assess the suitability of aspen wood shavings for the commercial cultivation of Oyster mushrooms.

The second aim of this study was to investigate some drying methods for the Phoenix Oyster mushroom, *Pleurotus sajor-caju*. Its rising importance in the exotic mushroom industry has created a need to find suitable postharvest methods for extending the shelf-life of the mushrooms. Oyster mushrooms lend themselves particularly well to dehydration because of their thin cross-section. However, there are still problems associated with the air-dried and reconstituted products such as brittleness and rubberiness respectively [Gormley and O'Riordain, 1976]. Freeze-drying is another potential method but it is expensive and although the dried product is of superior quality in appearance, the damage incurred by the freezing process on the tissue may adversely affect the texture of the rehydrated product. Oddson and Jelen [1981] observed that rehydrated freeze-dried *Pleurotus florida* mushrooms were very limp, probably more due to the inability of the delicate structure of the mushrooms to withstand the freezing process than the drying itself. Since the freezing step appears to be the main cause of quality impairment of freeze-dried mushrooms, the effects of bypassing that stage and only subjecting fresh mushrooms to the drying conditions usually used for freeze drying, i.e., heating under high vacuum, were investigated. *Pleurotus sajor-caju* mushrooms were air-, freeze-, and vacuum-dried. Parameters of interest were the microstructure, the rehydration properties, the textural characteristics, and the sensory evaluation of the products. The long term objective of this particular study was to assess the suitability of the high vacuum drying methods for higher value mushrooms such as the Chanterelles.

CHAPTER 2

CULTIVATION OF OYSTER MUSHROOMS

2.1 LITERATURE REVIEW

2.1.1 HISTORY OF CULTIVATION

Experiments on the cultivation of the Oyster mushroom have been reported since the beginning of this century. Falck [1917] found that Oyster mushrooms could be easily produced on sterilised straw and on tree stumps. Among early investigators who contributed to the understanding of the growing requirements for the Oyster mushroom are Busse, Etter and Kaufert [Weijer, 1974]. However, major innovations were made into Oyster mushroom cultivation by Block *et al.* [1958]. Pine and gum wood sawdust were supplemented with 5% oatmeal (dry weight basis), moistened, autoclaved for 30 minutes, inoculated with spawn of *Pleurotus ostreatus* and incubated at 25.5°C (78°F) and 85% relative humidity. Gum wood yielded 37% (g of fresh mushrooms per 100 g of dry substrate), while pine wood produced half that yield.

Bano and Srivastava [1962] experimented with paddy straw, rice hulls and horse manure for the cultivation of *Pleurotus ostreatus* and found that paddy straw was the best substrate and that supplementation with oatmeal gave the highest yield. In a later investigation, Bano *et al.* [1979] reported that compared to Ragi (*Eleucine coracana*) straw and wheat straw, paddy straw gave the highest yield as substrate for *Pleurotus flabellatus* mushrooms (460 g/kg of dry straw). All three substrates were supplemented with 5% horse gram (*Dolichus biflorus*) powder (dry weight basis). Kostadinov *et al.* [1971] reported that *P. ostreatus* was grown on crushed corn cobs under sterile conditions by Toth in 1969 and that Gyurko later modified that method using non-sterile conditions. Zadrazil [1978] reviewed the contribution of several researchers who laid the the foundation for the industrial production of *Pleurotus* substrate and discussed the cultivation requirements of *Pleurotus* species in detail.

Whereas research on the Oyster mushroom was initially focused on the species *Pleurotus ostreatus*, a wide range of *Pleurotus* spp. mushroms are now being studied for cultivation on an extensive array of suitable subsrates, which are considered as agricultural and industrial wastes. *P. florida*, originally considered as a strain of *P. ostreatus*, differs from the latter in some morphogical characteristics; it has a lower proportion of stems to caps and does not require a cold shock for induction of the fruitbodies [Oddson, 1979]. Jandaik and Kapoor [1974] reported the isolation of *P. sajor-caju* in India and successfully cultivated this species on banana pseudostems. Han *et al.* [1976] in Taiwan isolated *P. cystidiosus* , which they named "Abalone mushroom" because of the similarity of the cooked mushroom's texture to that of abalone. *P. flabellatus* is cultivated in India [Bano *et al.*, 1979] and in the Phillipines [Quimio, 1978].

Steinkraus and Cullen [1978] grew *P. ostreatus* on shredded paper supplemented with 5% wheat bran and 5% $CaCO_3$ (based on the dry weight of paper) and reported obtaining fresh mushrooms equivalent in weight to the original dry weight of substrate utilised. Tan [1981a] obtained a yield of 100% fresh *P. florida* mushrooms per dry weight of cotton waste supplemented with 5% calcium carbonate (dry weight basis). A mixture of waste paper, used tea leaves, wood ash and yeast tablets was found to be suitable to grow *P. sajor-caju* by Mehrotra *et al.* [1982] . Yamashita *et al.* [1983] found that peanut shells and lawn grass supplemented with 16% rice bran were as good as sawdust for the cultivation of Oyster mushrooms. They found that rice bran per se with porous stone as support gave yields ranging from 92% of dry rice bran weight. The porous stone could be recovered after and employed for the next cultivation. Mueller and Gawley [1983]

Gawley [1983] reported the successful cultivation of *P. sajor-caju* on a combination of pulp mill sludges and pomace (apple residue after pressing the pulp, including seeds and filtration aids). When supplemented with 3% soya flour (wet substrate basis), a maximum yield of 23.6% of fresh mushrooms (wet substrate basis) was obtained. Bischt and Harsh [1984] suggested an interesting way of dealing with the weed, *Lantana camara*. In some parts of India, this weed is overruning large cultivable areas because of its prolific growth and wide adaptability. It is also a source of fire hazards because it burns even when it is green. The authors found that a mixture of these weeds with waste paper in the ratio of 2:1 could be used to grow *P. ostreatus*. The weeds, however have to be chopped and boiled as part of the pretreatment. According to these authors, a net profit could be made using the weed as a substrate. Martinez *et al.* [1985] reported that fermented, pasteurised coffee pulp supported the growth of *P. ostreatus* .

The cultivation of the Oyster mushrooms is now expanding rapidly in several countries (Table 2). Hungary, Germany and Italy are the most important producers in Europe [Imbernon *et al.*, 1984] .

Table 2 Production of *Pleurotus* spp. mushroms in some countries

Country	**Tons/annum**	**Reference**
Japan	21,712	Mori, 1987
Italy	10,000	Lanzi, 1987
Germany	2,800	Botticher, 1987
Hungary	2,000	Koronczy and Stubnya, 1987

2.1.2 CLASSIFICATION

The *Pleurotus* mushroom can be classified as follows [Ainsworth, 1966; Smith, 1978]:

Division	Eumycota
Sub-division	Basidiomycotina
Class	Hymenomycetes
Order	Agaricales
Family	Tricholomataceae
Genus	*Pleurotus*

The Eumycota division groups organisms known as Fungi. The characteristics of Fungi include: possession of cell walls made of chitin (except in the class Oomycetes); lack of chlorophyll and a heterotrophic mode of nutrition. The diagnostic feature of the Basidiomycotina is the basidium, a club-shaped structure bearing the basidiospores. In the Hymenomycetes, the basidia are organised in a layer called the hymenium. The spores are exposed at all developmental stages and are violently discharged when mature [Dube, 1985]. The basidiocarp (fruiting body) of the Agaricales is fleshy with a stipe (stem) and a pileus (cap). In the Tricholomataceae, the pileus and stipe are confluent and the lamellae typically attached. Features for the genus *Pleurotus* include a lateral or excentric (between central and lateral) stipe, and smooth gill edge with usually white spores.

Pleurotus species differ from each other in morphological and physiological characteristics [Zadrazil, 1978] and even strains of the same species show variations. In addition, the appearance of the fruitbodies is influenced by environmental conditions; thus, the colour of the cap may be darker in outdoor conditions. The cap of *Pleurotus ostreatus* is shell-shaped, ranging from 5-15 cm in size. The colour varies from gray to gray-brown during fruitbody formation. The gills (lamellae) are broad, whitish, running down the excentric stipe [Zadrazil, 1978]. The fruitbodies often grow in overlapping shelving clusters. *P. sajor-caju* is simlar in shape to *P. ostreatus*. The colour varies from light brown to beige with a paler center. Unlike *P. ostreatus*, it is not essential for *P. sajor-caju* to undergo a cold shock for the induction of fruitbodies [Mueller and Gawley, 1983].

2.1.3 LIFE-CYCLE OF *P. OSTREATUS* AND *P. SAJOR-CAJU*

The life-cycle can be divided into two main stages: (i) reproductive and (ii) vegetative. The reproductive stage is represented by the fruitbody which produces basidiospores. When the spores land on a suitable substrate, they germinate into the vegetative hyphae. These hyphae form the primary mycelium and are monokaryotic (all the cells are uninucleate and are of the same genotype). Fusion of compatible hyphae is followed by the migration of nuclei from the monokaryon to the other to form a secondary mycelium. The result is the formation of a dikaryon whose cells contain paired nuclei of two different parental genotypes. The secondary mycelium under certain conditions is induced to form a primordium which eventually develops into a mature fruitbody. Within each basidium of the fruitbody, there is fusion of the paired nuclei followed by meiosis to produce basidiospores. Species exhibiting this particular pattern of life-cycle are referred to as heterothallic because fusion of monokaryotic mycelia is necessary to establish the fertile mycelium [Elliot, 1982]. The life-cycle is schematised in Figure 1.

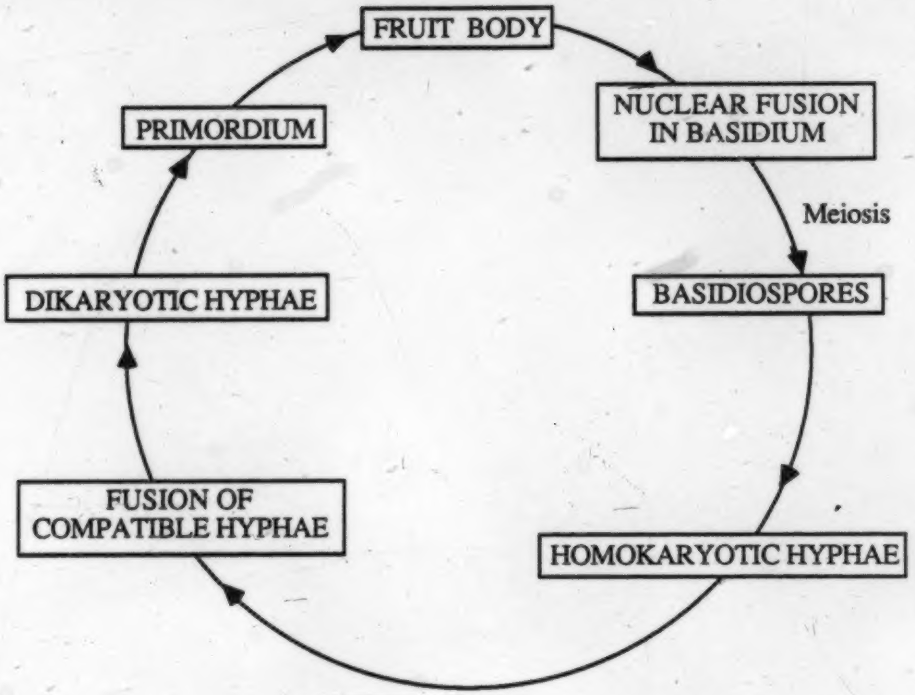
FRUIT BODY
NUCLEAR FUSION IN BASIDIUM
Meiosis
BASIDIOSPORES
HOMOKARYOTIC HYPHAE
FUSION OF COMPATIBLE HYPHAE
DIKARYOTIC HYPHAE
PRIMORDIUM

Figure 1. Schematic diagram of life-cycle of *P. ostreatus* and *P. sajor-caju*

2.1.3 NUTRITIONAL REQUIREMENTS OF *P. OSTREATUS* AND *P. SAJOR-CAJU*

P. ostreatus and *P. sajor-caju* are saprophytic organisms which grow on dead organic matter by secreting extracellular enzymes into the substrate and absorbing the hydrolysed nutrients back. Like most Fungi, they basically require: carbon (which can be obtained from polyssaccharides, cellulose, hemicelluloses, and sugars); nitrogen (either in inorganic form as nitrates or ammonium salts or as organic nitrogen compounds such as amino acids, peptides, or proteins); essential elements (e.g. phosphorus); and vitamins (e.g. thiamine) [Wood and Fermor, 1985]. The ability to utilise any compound and the minimal requirement for growth will, however, depend on the species, and for *Pleurotus* spp. knowledge of their nutritional requirements is still limited.

Carbon. In nature, *Pleurotus* spp. are found on dead trees and stumps, and are described as lignocellulolytic organisms because of their ability to utilise lignin and cellulose as carbon sources [Zadrazil, 1978]. For cultivation purposes, however, a range of cellulosic materials has been found to be suitable [Kurtzman and Zadrazil, 1982]. In addition to lignin and cellulose, *P. ostreatus* and *P. sajor-caju* can utilise other carbon sources such as starch and sugars. Hashimoto and Takahashi [1976] investigated the effects of a range of carbon sources on the mycelial growth of *P. ostreatus*. They observed that the greatest growth was supported by mannose followed by starch, glucose, maltose, sucrose, fructose, mannitol, ethanol, and galactose. Xylose, arabinose, citrate, malate, fumarate, succcinate, oxalate supported less growth but this was still from four to five times better than the control. Jandaik and Kapoor [1976a] studied the ability of *P. sajor caju* to use various sugars and sugar alcohols. Starch was found to support the best growth but good growth was also obtained from maltose, glucose, sucrose and dextrin. Mannitol, sorbitol, xylose and arabinose supported poorer growth. Kurtzman and Zadrazil [1982] cautioned against using the smaller carbohydrate compounds since they would provide a readily available source of carbon sources for undesirable competing organisms. They advised to remove all carbohydrates other than cellulose from the substrate by a wash pasteurisation to avoid contamination by weed fungi.

Nitrogen. As for all other organisms, *P. ostreatus* and *P. sajor-caju* require a source of nitrogen but it seems that their requirement is minimal [Kurtzman and Zadrazil, 1982]. In nature, they will grow on substrates with a very low N:C ratio such as wood. This prompted the speculation that these organisms may have the capacity to fix nitrogen from the atmosphere [Ginterova and Maxianova, 1975]. Rangaswami *et al.* [1975] showed a steady increase followed by a decline of the nitrogen content of the substrate they inoculated with *P. sajor-caju*. They attributed this nitrogen gain to the nitrogen-fixing capacity of *P. sajor-caju*. Zheng Shi-Li [1984], however, found that *P. sajor-caju* was incapable of fixing nitrogen and that the increased nitrogen content observed during *Pleurotus* cultivation was the result of the activity of various nitrogen-fixing bacteria. Studies on the supplementation of nitrogen sources to the substrate have shown that depending on the nature and the level of the nitrogen source, an improvement in the yield of the *Pleurotus* mushrooms could be obtained. Jandaik and Kapoor [1976b] found that among several ammonium salts, nitrates and urea, the latter supported the greatest mycelial growth for *P. sajor-caju*. Zadrazil [1980] studied the effects of different concentrations of ammonium nitrate (nitrogen content of 4.18%, 4.32%, 4.78%) on the yield of *P. sajor-caju* fruitbodies. There was an increase in yield with the first and second level of supplemmentation but at the highest level the yield was even lower than the control. Hashimoto and Takahashi [1976] found that ammonium tartrate promoted better mycelial growth than ammonium nitrate. The use of inorganic fertilisers, however, is expensive and for most mushroom growers, supplementation with an organic source is preferred if it is available locally and is inexpensive. Grass meal (ground grass), soya meal, chicken feathers' flour, rice bran, and canola meal have all been found to increase the yield of *Pleurotus* mushrooms [Kalberer, 1976; Laborde *et al.*, 1984; Mueller and Gawley, 1983; Sosulski, 1985].

Minerals. Information on the mineral requirement of *P. ostreatus* and *P. sajor-caju* is scant. Furthermore, studies in this area are complicated by the fact that it is difficult to obtain media free from any impurity [Kurtzman and Zadrazil, 1982]. Hong [1978] found that potassium dihydrogen phosphate increased the growth of *P. ostreatus* significantly. According to Kurtzman and Zadrazil [1982], it is the phosphate which is the important nutrient. Jauhri and Sen [1978a] compared several phosphate sources and found that urea phosphate promoted the best growth in *P. ostreatus*. The need for magnesium sulphate was reported by Hong [1978] and Jauhri and Sen [1978b]. Zinc (as zinc sulphate) and iron (as ferric sulphate) were also required for optimum growth [Jauhri and Sen , 1978b]. Kurtzman and Zadrazil [1982] argue that there was no evidence that the anions, *i.e.* sulphate did not participate in the effect. Jauhri and Sen [1978b] also raised the possibility of iron impurities entering the medium from chemicals, glassware and water, so that the essentiality of the iron could not be conclusively established.

Vitamins. All the water-soluble vitamins tested have been found to stimulate the growth of *P. ostreatus* but thiamine was found to promote more growth than any other vitamin. Thus when other vitamins were added to the basal media containing thiamine, no additional growth occurred [Kurtzman and Zadrazil, 1982]. Hashimoto and Takahashi [1976] found a small increase in mycelial growth when biotin (10µg/L), niacin (30µg/L), or riboflavin (50µg/L) were added to a basal liquid medium containing thiamine at 1.0mg/L but a decrease when pyridoxine or pantothenic acid (50µg/L) were added. With 100µg/L of thiamine in the medium, mycelial growth was comparable to that obtained from 1mg/L of thiamine with (10µg/L) of biotin together. The authors concluded that only thiamine was required for growth and that the addition of other vitamins did not influence growth significantly. Jandaik and Kapoor [1976] studied the effects of six water-soluble vitamins on the growth of *P. sajor-caju*. Maximum growth was obtained when all the vitamins were added to the basal medium. A significant decrease in growth was observed only when thiamine or biotin were omitted. The optimum dose of thiamine or biotin were found to be respectively 50µg/L and 10µg/L.

2.1.4 CULTIVATION STEPS

The basic cultivation steps for the Oyster mushroom are summarised in Figure 2.

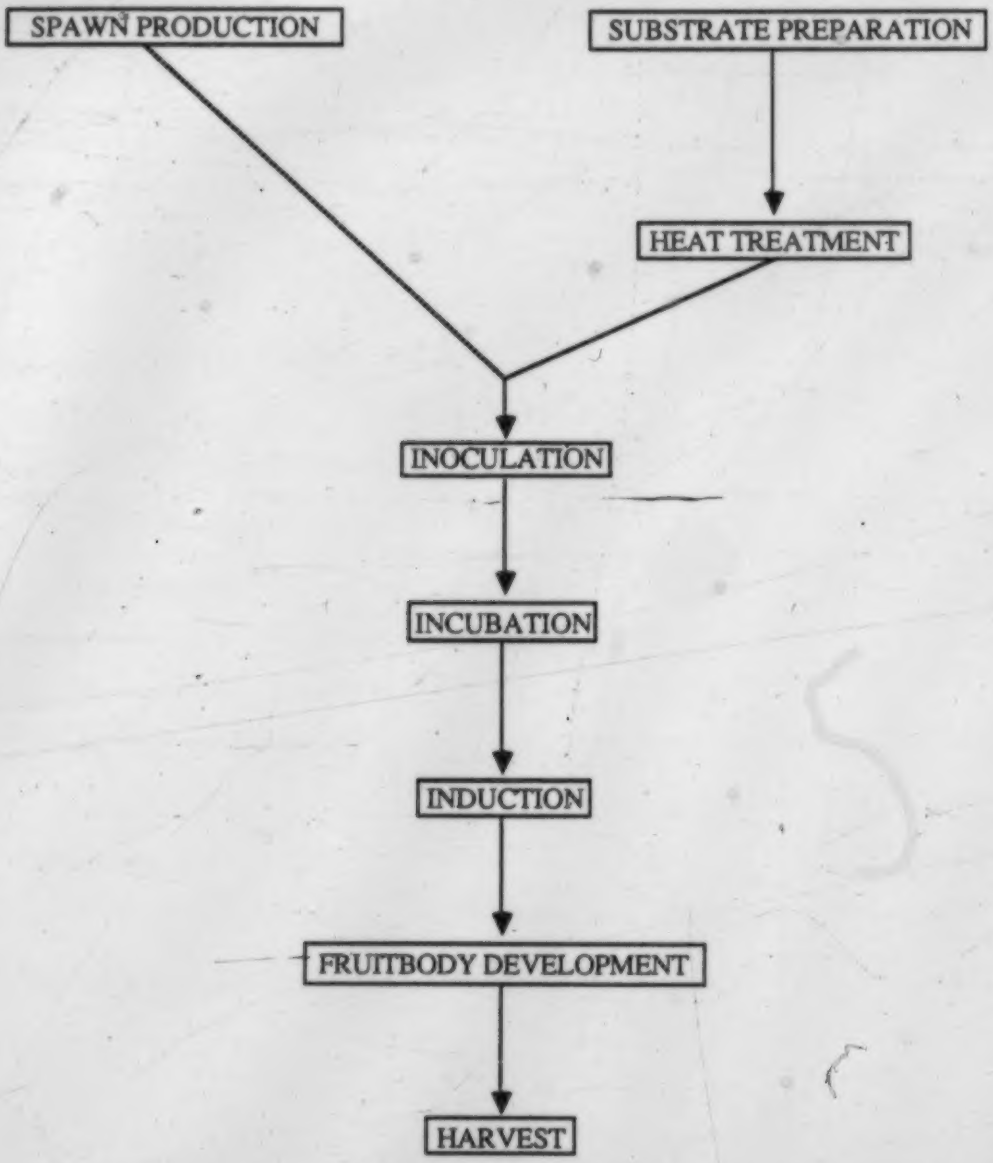
SPAWN PRODUCTION
SUBSTRATE PREPARATION
HEAT TREATMENT
INOCULATION
INCUBATION
INDUCTION
FRUITBODY DEVELOPMENT
HARVEST

Figure 2. Cultivation steps in *Pleurotus* production (optimum environmental conditions at each stage vary with different species).

Spawn production. Spawn consists of dense mycelium growing on a medium (e.g. wheat kernels). The spawn is used for propagating the mycelium into the substrate used for cultivation. For the preparation of grain spawns (e.g. rye, sorghum, wheat), the following method is recommended by Chang [1982]. One hundred grams of grain, 150 mL of water and 2 g chalk in a 500 mL flask are first sterilised, inoculated with a pure culture under aseptic conditions, then incubated until the mycelium has grown throughout the medium. A variety of materials can be used for spawn preparation. They include: millet, finely shredded straw, perlite coated with wheat flour, rice and/or wheat bran [Weijer, 1974], as well as used tea leaves, cotton waste, or coffee pulp [Chang, 1982]. Kostadinov *et al.*, [1971] investigated the use of a liquid spawn. The mycelium was grown as a submerged culture in a nutritive medium containing 5% cane molasses, 0.3% ammonium nitrate, 0.1% ammonium sulphate, 0.1% potassium phosphate in 100 mL of solution. They compared the growth of the mycelium from the liquid spawn and from a solid spawn (sterile corn cobs) through the substrate of corn cobs and found that the liquid inoculum spread through the substrate in 22 to 30 days while the solid inoculum took a period of 21 to 26 days. Since a liquid spawn could be cultured on a continuous basis, it could be produced in a shorter period of time. However, its application on a large scale is limited by the high cost of production [Zadrazil and Kurtzman, 1982] and the efficiency of liquid inoculum still needs to be investigated further.

Substrate. If the substrate is straw or any other large-sized material, it is first shredded into smaller pieces. The substrate is then soaked in water to bring the moisture content to a higher level, the optimum for *Pleurotus* being about 70% wet weight [Zadrazil and Kurtzman, 1982]. The substrate is then heat-treated to prevent the growth of competing microorganisms. Heat treatment can be in the form of fermentation, pasteurisation or sterilisation. Sterilisation by autoclaving with steam at 121°C at 138 kPa (20lb/in^2) ensures that all microorganisms in the substrate are destroyed so that only the inoculated species can grow in this medium. Inoculation should be carried out under aseptic conditions, and incubation should be under strictly hygienic conditions. Any contamination of the uncolonised sterilised substrate would lead to a rapid spread of the undesirable organism.

Pasteurisation can be carried out by different techniques which in turn depend on the type of substrate being used. Zadrazil and Schneidereit [1972] suggested a temperature of 72°C for 4-5 days. Stanek [1976] recommended a pasteurisation proceedure of 50-70°C for 1-2 days. According to Visscher [1984], a pasteurisation temperature of 70°C is commonly used in Northern Italy, the main producing area in Europe but he showed that such a temperature may affect the yield of mushrooms negatively. He found that a substrate pasteurisation temperature of 58°C followed by an incubation temperature of 25°C for mycelial growth gave a better yield than substrate pasteurised at 70°C. Laborde [1983] cautioned against using a high temperature for pasteurisation as the chance of contamination by *Trichoderma* could become greater. Above a temperature of 60°C and under very humid conditions, part of the microflora could be destroyed leaving more opportunities for *Trichoderma*, an important competitor of *Pleurotus*, to grow when the temperature goes down [Laborde *et al.*, 1984]. A pasteurisation at 45°C for 72 hours followed by a conditioning treatment of 58°C for 5 hours was suggested by Flick-Saalbaach [Visscher, 1984] to keep weed moulds to a minimum during mycelial growth. According to Chilton [1984], one method used in Hungary consists of exposing the sustrate to a temperature of 60°C for 6-12 hours using live steam. The temperature is then lowered to 50°C for 36-48 hours. The first phase is believed to kill the harmful competitors and insects while the second conditioning phase gives the substrate a high degree of selectivity which helps to prevent contamination throughout the crop. This selectivity is probably due to the presence of thermophilic microorganisms which have proliferated during the second phase. Laborde *et al.*, [1984] recommended that the temperature of the substrate be maintained at 60-61°C for 12-18 hours to achieve proper pasteurisaton. The temperature should then be allowed to fall to 25-30°C after which inoculation can be carried out. Kurtzman [1982] argued that steaming straw for pasteurisation was not a reliable method because of the possibility of pockets which may remain cold, harbouring insect eggs, nematodes and unwanted fungi and bacteria. Instead he suggested a wash pasteurisation techique which consists of soaking the substrate in hot water (65 ±5°C) for 10 minutes to 1 hour [Kurtzman, 1975]. An alternative method of heat treatment was reported by Balazs [1984]. The substrate (straw, maize cobs) was heated in the dry state at 100°C for 1 hour followed by moistening and mixing with grain spawn in one run. This method is used in conjunction with small amounts of chemicals to control competing organisms.

Fermentation is the least costly method of heat treatment. The substrate is piled up, kept moist and turned every one or two days for a few days. A microbial succession occurs in the substrate during which there is the breakdown of the substrate into a compost. Heat is generated during the process and the growth of the thermophilic microorganisms is favoured [Chang and Miles, 1982]. The presence of these organisms protects the substrate from contamination by pathogenic competitors. The inoculation of the substrate with *Streptomyces thermovulgaris* and *Pseudomonas* spp. to ferment the substrate has apparently been reported by Stanek and Rysava [Zadrazil and Kurtzman, 1982]. Martinez *et al.* [1985] obtained a good yield of *P. ostreatus* (132 g/kg dry weight substrate) from coffee pulp fermented for five days, followed by a short pasteurisation (immersion in hot water for 15 minutes). The fermentation process in this case also brought the of caffeine in the pulp, so that the spent substrate could be considered for ruminant feed. Compared to heat-treated substrate, fermented substrate is less susceptible to infections but there is a dry weight loss of 8-15% during the fermentation process [Kalberer, 1976].

It is usually the practice of many growers to add to the substrate a buffer such as calcium carbonate to stabilise the pH to what is believed to be the optimum. However, as reviewed by Kurtzman and Zadrazil [1982], there is no total agreement on the optimum pH. Depending on nature of the substrate, the optimum pH reported for *Pleurotus* ranged from 4-6.5. Mycelial growth changes the pH value of the substrate and there is evidence that the organism will tend to lower or raise the initial pH to settle near the optimum. Tan [1981b] found that the addition of up to 20% of calcium carbonate did not change the pH of cotton waste substrate to any significant extent. He also observed that there was no difference in mushroom yield between the substrate with and without calcium carbonate.

Inoculation. The rate of spawning is a factor influencing the yield of the fruitbodies. Laborde *et al*. [1984] studied the yield of *P. pulmonarius* from straw spawned at the rate of 2%, 5% and 8% (wet weight of substrate). A yield of 12.9%, 16.2% and 18.2% (fresh mushrooms per inoculated substrate) respectively was obtained. A higher inoculation rate was accompanied by a higher increase in the the temperature of the substrate. With a larger inoculum, care has to be taken so that the temperature of the block of substrate does not reach a level lethal to the mycelium. Zadrazil [1978] found that when higher quantities of inoculum were used, the temperature and carbon dioxide concentration increased more quickly. Commercially, a spawning rate of 0.5% to 5% of wet weight of substrate is being used [Zadrazil and Kurtzman, 1982].

Incubation. Once the heat-treated substrate has been inoculated, it is incubated to allow mycelial growth. The time of incubation depends on several factors such as the optimum temperature for the species and the strain, the nature of the substrate, and the rate of spawning. Mueller and Gawley [undated] incubated *P. sajor-caju* at 16°C and 24°C. At the lower temperature, it took longer for the mycelium to grow through the substrate and for the fruitbodies to develop but the yield of the first flush was 25% higher than that at 24°C. A range of growth systems is being used for incubating the substrate. Polyethylene bags are commonly used by many European growers who have a fully mechanised system for spawning and filling the bags [Zadrazil and Kurtzman, 1982]. Although the mycelial growth is stimulated by high carbon dioxide level, oxygen must still be supplied to the substrate [Zadrazil, 1978]. Garcha *et al.* [1985] reported a higher yield of mushrooms from perforated rather than from unperforated polyethylene bags. They also compared three different sizes of bags (17"x 12", 19.5"x 16", 25"x 17") and found no significant effect on yield. The small sized bags would be easier to handle and would make better efficiency of indoor space. The temperature of the substrate in the bag should not be allowed to exceed the lethal level of 33°C. One method devised by Zadrazil [1978] consists of a removable cylindrical plastic case (2 m high with a radius of 30 cm) and a central metal rod with a basal metal plate to support the developing cylinder. The central tube helps to lead the heat off the center. This system offers the advantage of a large surface area to volume ratio. Another method is by packing the inoculated substrate into a polyethylene plastic foil and shaping it into a rectangular block by a wooden mould (30 cm x 30 cm x 60 cm). The blocks can be stacked (5-8) on metallic shelves with wheels for an efficient use of space and for mobility [Laborde *et al.*, 1984].

Induction. When the mycelium has permeated through the substrate, the formation of fruitbodies is initiated by the process of induction. The fruitbodies first appear as mycelial aggregations known as primordia which later differentiate into a stipe (stalk) and a pileus (cap) [Hayes, 1985]. The induction process consists of altering the climatic conditions surrounding the substrate. Temperature, light, air humidity, and air composition (CO_2 and O_2) are important factors, the optimum values of which will depend on the species and the strain concerned. For *P. ostreatus*, the surrounding temperature is lowered to below 15°C for at least 12 hours, lighting of at least 150 lux or more is provided for at least 8 hours daily, the humidity of the air is raised to 85-95% level and the CO_2 level is maintained below 0.1% [Laborde, 1981, 1984]. For *P. sajor-caju*, the requirements are similar except that no temperature shock was found to be necessary [Mueller and Gawley, 1983]. Imbernon *et al.* [1984] reported that the Oyster mushrooms can be broadly classified according to their fruiting temperature requirement as the cold strains (5°C to 15°C) and the hot strains (15°C to 25°C). If the growth containers are bags, they are usually slit open but if the bags are already perforated, the fruitbodies will grow through the exposed areas. If prior to incubation, the substrate was not packed properly in the bag so that there would be some space between the surface of the block and the plastic bag, then during induction, a mass of primordia could form in that region but would not develop further. One way to avoid this problem would be to use the method described by Chakravarty and Sarkar [1984]. For incubation, the substrate is placed in a nylon-net bag which is covered with a perforated polyethylene bag. As soon as the primordia appear following induction, the polypropylene bag is removed.

Fruitbody development. For normal fruitbody development and appearance, temperature, lighting, humidity and ventilation have to be carefully controlled according to the strain's requirement. Zadrazil and Schneidereit [1972] reported on the distorted appearance of *P. ostreatus* fruitbodies allowed to develop in extreme climatic conditions. With insufficient lighting, the stipe was longer and thicker and the size of the pileus smaller. A high level of CO_2 (1-2 vol%) and lack of light resulted in the formation of a deformed mass of highly branched growth. Imbernon *et al.* [1984] found that *P. pulmonarius* took only 2 days to reach maturity at 20°C compared to 6.2 days at 12°C. However, at the lower temperature the fruitbodies were darker and fleshier. Block *et al.* [1959] found that *P. ostreatus* fruitbodies grew normally when the relative humidity of the atmosphere was 75-85% but at a higher R.H. level (95-100%) the stipes became unusually long and the caps were small and in a closed condition. The appearance of the fruitbody is also influenced by the orientation of the surface on which it is growing; thus on beds the fruitbodies are said to develop central stems while on walls the stems are eccentric or lateral. Mueller and Gawley [undated] found that the number, size and appearance of *P. sajor-caju* fruitbodies were influenced by the surface available for fruiting. A limited surface area (pots which only had holes in the side and the top surface as the exposed area) favoured larger fruitbodies with a tendency to grow in clusters. If the block of substrate was completely removed from its container thereby exposing a large suface area, there was a larger number of smaller sized single fruitbodies.

Harvest. Fruitbodies are usually harvested before they reach maturity (when the edges start to curl up). Harvesting at a later time will increase the chances of exposure to an atmosphere fully laden with spores which may cause allergic reactions in some people. Fruitbodies in a particular cluster are not all at the same stage of maturity and this may complicate the picking work [Imbernon *et al.*, 1984]. Brooke-Webster and Cairns [1987] described an interesting method to pick all the fruitbodies from a cluster efficiently. The substrate was covered by a thin polyethylene film (0.02 mm) perforated with holes. This film was overlaid by another unperforated plastic sheet which was removed during induction. The first layer adhered very closely to the substrate thereby limiting the fruitbody development only in the exposed area. The clusters growing through the holes were cut at the base. This method allowed the fruitbodies to fall individually on the clean plastic sheet where they can easily be picked.

Although there is general agreement on the basic steps involved in the cultivation of the Oyster mushroom, the technology employed by any particular grower will be determined by geographical and economic considerations.

2.2 CULTIVATION EXPERIMENTS

2.2.1 OBJECTIVES

The aim of the cultivation experiments was to assess the suitability of Aspen wood shavings, using as supplementations waste products from the local agricultural and food industries, to grow Oyster mushrooms, *Pleurotus ostreatus* and *Pleurotus sajor-caju*. Liquid cheese whey, a waste product of the dairy industry, contains a fair amount of nitrogen in the form of whey protein and carbohydrate as lactose [Bernal and Jelen, 1985]. Duvjnak *et al.* [1978] explored the use of liquid whey for the submerged culture of *Morchella hortensis* and *Agaricus campestris* mycelium. The use of whey as a soaking medium for solid substrate for mushroom cultivation, however, has not been reported. Canola meal is a by-product of the canola oil extraction process, a major industry in Western Canada. It is rich in nitrogen and has been identified by Sosulski [1985] as a good supplement to be added to wheat straw for cultivating *Pleurotus sajor-caju* mushrooms.

Allergies to the spores of *Pleurotus* spp. have been reported (Zadrazil and Kurtzman, 1982). After having been exposed to the spores of *Pleurotus* spp. mushrooms during harvesting , some people experienced general fatigue, headache, coughing and fever. In response to this problem, sporeless strains of *Pleurotus* spp. have been isolated [Eger, 1977; Chang *et al.*, 1985]. However there is little information on the yield and suitability of the sporeless strains for cultivation. This study included an experiment on a normal and a sporeless strain of *Pleurotus ostreatus*, obtained from a commercial European spawn maker.

The objectives of this study were:

1. To determine the yield of fresh Oyster mushrooms on aspen shavings per se.
2. To evaluate the use of liquid cheese whey as a soaking medium and a source of nutrients in aspen shavings substrate.
3. To compare the yield of fresh mushrooms from aspen shavings supplemented with soya flour and canola meal and to investigate the effects of different levels of canola meal supplementation in aspen on the yield of fresh mushrooms.
4. To assess the suitability of a sporeless strain of *Pleurotus ostreatus* for cultivation and to verify the absence of spores in that strain by scanning electron microscopy.

2.2.2 METHODOLOGY

Five cultivation experiments were carried out. The first cultivation experiment compared the yield of a normal and a sporeless strain of *Pleurotus ostreatus* on Aspen shavings supplemented with liquid cottage cheese whey. The other four cultivation experiments dealt with *Pleurotus sajor-caju* on Aspen shavings with different sources of supplementation, i.e, liquid cottage cheese whey, soya flour and canola meal.

2.2.2.1 Cultivation experiments on *Pleurotus ostreatus*

Strain

A normal strain (P_2) and a sporeless strain (Sp_2) of *Pleurotus ostreatus* were obtained from Hauser Champignonkulturen, AG, Gossau-Zurich, Switzerland.

Substrate

Aspen shavings were supplied from a local sawmill, Sunchild Forest Products, Edmonton, Alberta. Liquid cottage cheese whey was from Palm Dairies, Edmonton, Alberta.

Spawn preparation

Five hundred mL flasks of spawn were prepared. One hundred grams of rye grains with 150 mL of water and 2 g of chalk were placed in the flask and autoclaved for 1 hour at 121°C. After being cooled to room temperature, the grains were inoculated with the culture and incubated at 25°C for three weeks.

Growth containers

Polyethylene bags (30 cm x 61 cm) were used as growth containers for the myceliation stage. For the fruiting stage, bags were removed and the blocks of substrate were surrounded with wire mesh (Plate 1).

Growing environment

Cultivation was carried out in the growth chambers of the Botany department, University of Alberta.

Method

The experiment was designed as a randomised block design with 3 factors, each at 2 levels: (i) normal or sporeless strain; (ii) substrate soaked in water or whey, (iii) substrate pasteurised or non-pasteurised. There were 4 blocks with 8 experimental units per block. The cultivation method of Zadrazil (1978) was followed in general.

Substrate preparation

Half of a batch of shavings was soaked overnight in tap water (substrate Y) and the other half in whey (Substrate X). Shavings were allowed to drain for 6 hours and each type of substrate was packed in 4 polyethylene bags (3.75 kg wet substrate per bag). Two bags containing substrate Y and 2 bags containing substrate X were subjected to a heat treatment of 70°C for 18 hours and allowed to cool to room temperature. The other 4 bags were left unpasteurised. This proceedure was repeated four times for four different batches of shavings.

Inoculation and mycelation

Half of the bags with substrate Y and half of the bags with substrate X from each batch were inoculated with the P_2 spawn while the other bags with the Sp_2 spawn. The rate of spawning was 3% w/w (wet substrate basis). The bags were incubated without light for 30 days at 24°C, after which the shavings were permeated with the white mycelial growth.

Induction and fruiting

The temperature was lowered to 11°C and the relative humidity of the room brought to 95%. The bags were removed and the blocks of substrate were surounded with wire mesh. Bags heavily contaminated with mold were discarded at this stage. Cool white fluorescent light was provided to give 200 lux of light continuously at the surface of the substrate and carbon dioxide level was maintained around 500 ppm. Primordia started to appear 10 days after start of induction period. For fruitbody formation, the temperature was raised to 15°C while the relative humidity was kept at 85-90%. Light intensity was increased to 500-1000 lux and light was provided for 12 hours daily. The blocks of substrate were kept moist by watering twice daily.

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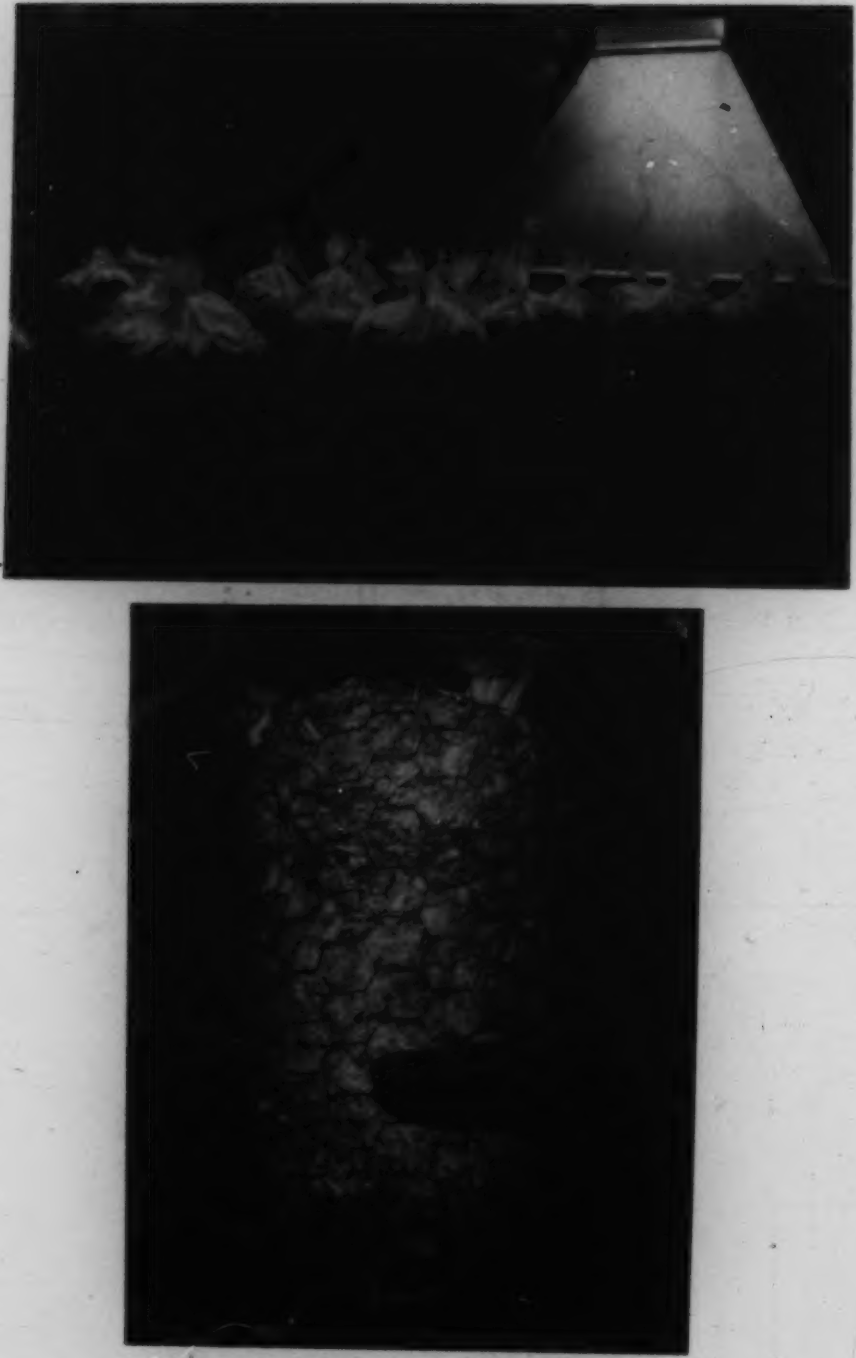


Plate 1 Cultivation experiment on *Pleurotus ostreatus*.
A-Incubation in plastic bags
B-Block of substrate with bag removed and surrounded by wire mesh for induction and fruitbody development

2.2.2.2 Cultivation experiments on *Pleurotus sajor-caju*

Strain and spawn

Pleurotus sajor-caju, ATCC Strain 32078, was obtained from Advanced Biotechnology Ltd., Vancouver, Canada. Spawn was prepared as described for *Pleurotus ostreatus* spawn (2.2.2.1).

Materials

Aspen shavings were supplied from a local sawmill, Sunchild Forest Products, Edmonton, Alberta. Three different supplements were used: liquid cottage cheese whey from Palm Dairies, Edmonton, Alberta; soya flour from High Level Natural Foods, Edmonton, Alberta; defatted canola meal from Western Canadian Seed Processor Ltd., Lethbridge.

Growth containers

For experiment 2, two and a half litre plastic flower pots were used (Plate 2). The pots were 15.5 cm high with a bottom diameter of 15 cm and a top diameter of 20 cm. There were four 1 x 1.5 cm drainage holes on the side wall near the bottom, and 1.2 cm diameter opening in the bottom. For experiments 3-5, aluminium trays were used. The trays were 6 cm high; the bottom surface was 26 x 21 cm, and the top surface, 30 x 23 cm. The bottom surface was perforated with 25 holes of 0.5 cm diameter.

Growing environment

For experiments 2, 3, and 5, a computer-regulated growth cabinet (Conviron PGW 36, Conviron Ltd., Winnipeg) in the Plant Sciences Department , University of Alberta was used. For experiment 4, a growth chamber in the Plant Sciences Department, University of Alberta was used.

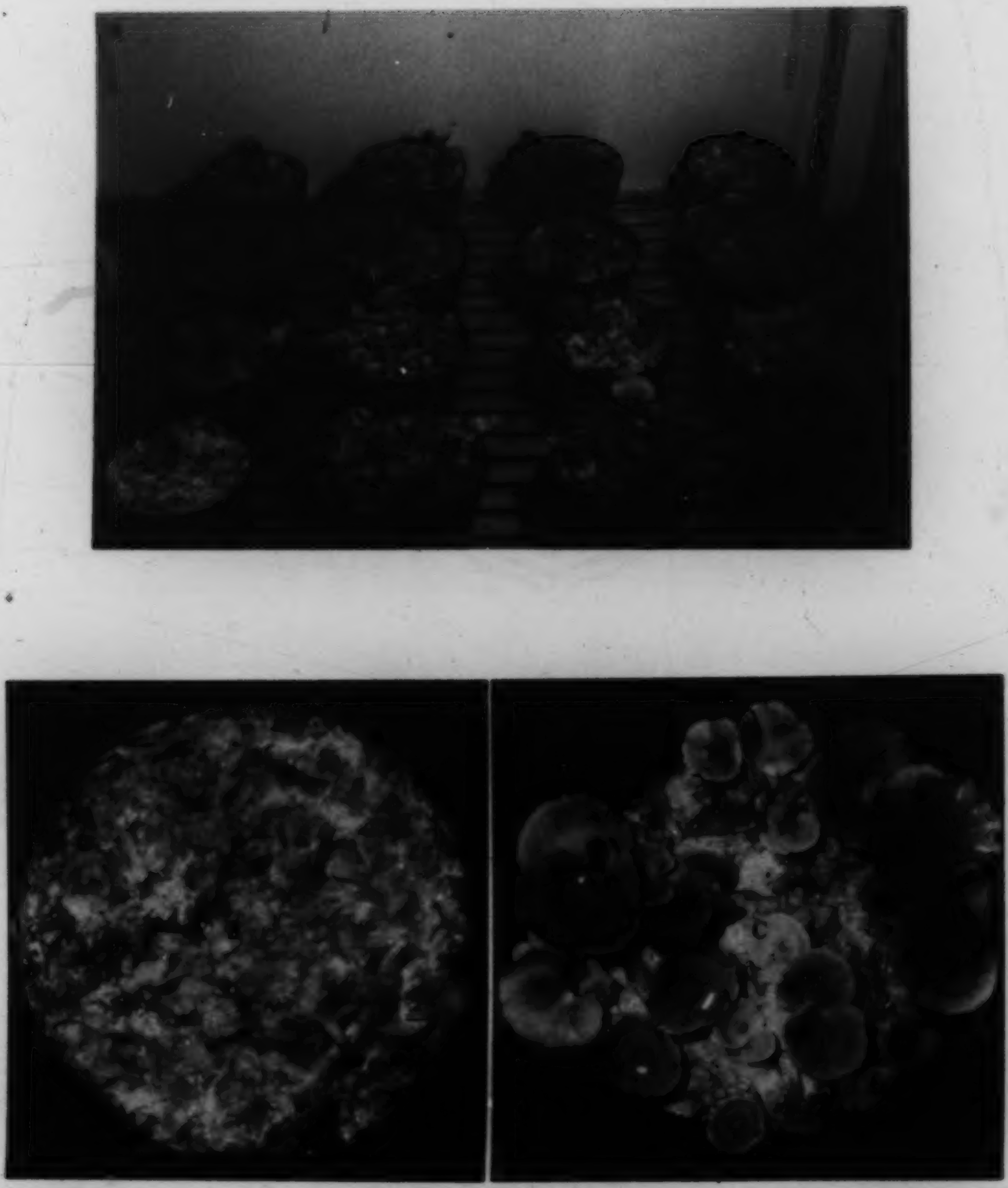


Plate 2 A-Set up for cultivation experiment 2 on *P. sajor-caju*
B-Mycelial growth. C-Fruitbodies before harvest

Substrate preparation

Seven hundred and fifty grams of shavings were soaked overnight in 8 litres of water or whey (in experiment 2) in a plastic bag. The shavings were drained on a perforated tray for 4 hours. For experiments 4 and 5, a source of supplementation was added (Table 3) and 1 kg of the wet substrate was weighed into a growth container unit. The plastic pots in experiment 2 were enclosed in a polyethylene bag while the aluminium trays in experiments 3-5 were covered with aluminium foil. The number of replicates used for each experiment is given in Table 3.

Table 3 Substrates used for cultivation experiments 2-5

Cultivation experiment	Replicates	Substrate
2	10	Water-soaked shavings
	10	Whey-soaked shavings
3	7	Water-soaked shavings
4	7	Water-soaked shavings with 3% soya flour *
	7	Water-soaked shavings with 3% canola meal*
5	5	Water-soaked shavings with 0% canola meal*
	5	Water-soaked shavings with 3% canola meal*
	5	Water-soaked shavings with 5% canola meal*
	5	Water-soaked shavings with 7% canola meal*
	5	Water-soaked shavings with 10% canola meal*

*(wet substrate basis)

Heat treatments

The enclosed plastic pots for experiment 2 were placed in a dry pasteurisation chamber and subjected to the following regime: pasteurisation at 70°C for 18 hours followed by a conditioning treatment of 45°C for 48 hours. The covered aluminium trays in experiments 3-5 were sterilised in an autoclave at 121°C for one hour.

Inoculation and myceliation

After being allowed to cool, each unit of substrate was inoculated with spawn at the rate of 3% w/w (wet substrate basis) and then incubated at 25°C without any light for two weeks. The plastic pots remained enclosed within the polyethylene bags. The aluminium trays had the aluminium foil removed and were enclosed within polyethylene bags.

Induction and fruiting

The containers were removed from their bags and the temperature was brought down to 20°C. The surface of the substrate was exposed to 200 lux of light from cool fluorescent lamps for 12 hours daily. The carbon dioxide level was maintained at 500 ppm by ventilation and the relative humidity was kept at 85-90%. The substrate was kept moist by watering twice daily. The primordia appeared within 2-3 days; fruitbodies matured in 3-5 days.

2.2.3 MEASUREMENTS AND ANALYSES

2.2.3.1 Yield

Fruitbodies were considered mature when the edges of the caps just started to curl up. They were picked at individual maturity. Yield was calculated as amount of fresh mushrooms (g) per kg of wet substrate.

2.2.3.2 Moisture content and pH of substrate

For moisture determinations, about 10 g samples of shavings were dried at 105°C to constant weight (76 hours). Determinations were made in triplicates.

A 20 g sample of shavings was soaked in 40 mL of water for 3 hours and the pH was then measured using a digital pH meter (Orion, model 601A).

2.2.3.3 Carbon to nitrogen ratio of substrate

The % carbon and % nitrogen content of the shavings, whey-soaked shavings (dry matter) and canola meal were analysed using an Elemental analyser (The Perkin-Elmer Model 240) at the Microanalytical Laboratory, Chemistry building, University of Alberta. The amount of carbon and nitrogen in the shavings supplemented with different levels of canola meal was then calculated.

2.2.3.4 Nitrogen content of substrate and mushrooms

Samples of substrate and mushrooms were dried to constant weight and analysed for nitrogen content by the micro-kjeldahl method (A.O.A.C., 1984, no. 47.021).

2.2.3.5 Texture analysis

The textural properties of the mushrooms were measured by the Ottawa Texture Measuring System [Voisey, 1971]. A 20 cm^2 wire food cell was used and the deformation rate was 12.5 cm/min. Mushrooms were rehydrated and cut into pieces of similar size and samples of 25 g were placed inside the cell. The force required to shear and extrude the mushrooms through the cell was recorded and used as an indicator of the product's toughness or tenderness.

2.2.3.6 Ratio of cap to stem

Samples of mushrooms harvested on any one day were weighed, their caps and stems separated and weighed separately. The ratio of the weight of the caps to that of the stems was then calculated.

2.2.3.7 Scanning electron microscopy

Samples were prepared according to the method described by Nickerson *et al.* [1974] to observe fungal spores. Small pieces of stipe and gill tissue ($5mm^2$) of fresh mushrooms were fixed by osmium tetroxide vapour and air-dried at room temperature. The dried samples were then sputter coated with gold, and examined with the scanning electron microscope (Cambridge model S250) at 20 kV.

2.2.4 RESULTS AND DISCUSSION

The substrate characteristics for each cultivation experiment are given in Table 4.

Table 4 Substrate characteristics for each cultivation experiment

Experiment	Substrate	% moisture	Heat treatment	pH	% Nitrogen (dry matter)
Cultivation 1	water-soaked shavings	69	pasteurisation	6.8	0.10
	whey-soaked shavings	72	pasteurisation	4.9	0.30
Cultivation 2	water-soaked shavings	71	pasteurisation	6.6	0.07
	whey-soaked shavings	69	pasteurisation	4.7	0.42
Cultivation 3	water-soaked shavings	72	sterilisation	6.3	0.11
Cultivation 4	shavings with 3% canola meal	71	sterilisation	5.9	0.61
	shavings with 3% soya meal	72	sterilisation	6.0	0.52
Cultivation 5	shavings with 0% canola meal	72	sterilisation	6.3	0.10
	shavings with 3% canola meal	71	sterilisation	6.0	0.61
	shavings with 5% canola meal	71	sterilsation	6.0	0.81
	shavings with 7% canola meal	69	sterilisation	6.1	1.11
	shavings with 10% canola meal	68	sterilisation	6.0	1.62

2.2.4.1 Yield of *Pleurotus* mushrooms on Aspen shavings

The yield of mushrooms on aspen shavings per se was low. The yield (first flush) of *P. sajor-caju* from the sterilised substrate was 3.2% (Table 5). Mueller and Gawley [1983] reported a similar yield of 2.9 % (wet substrate basis) of *P. sajor-caju* on sterilised pulp mill sludges. Aspen is a hardwood made up of about 56% cellulose, 16% hemicellulose, and 16% lignin [Wenzl, 1970] and is poor in nitrogen with a content of 0.07-0.1% (Table 4). Being lignocellulolytic, *Pleurotus* mushrooms will grow on wood sources but the nutrient content of the aspen substrate was inadequate to produce a significant quantity of mushrooms.

Table 5 Yield of *Pleurotus sajor-caju* (first flush) mushrooms on sterilised aspen shavings (g/100g wet substrate inclusive of spawn)

Replicate	Yield from first flush
1	2.84
2	4.24
3	4.51
4	1.49
5	3.61
6	2.61
7	3.20
Mean(±S.E)	3.21(±0.39)

2.2.4.2 Growth of *Pleurotus* mushrooms on whey-soaked shavings

The yield of *P. sajor-caju* mushrooms was increased by more than two-fold when the shavings were soaked in whey instead of water (Table 6). The main components of cottage cheese whey are 93.62% water, 0.75% protein and 4.90% lactose [Bernal and Jelen, 1985]. The increased yield observed could be partly due to the addition of the proteins from the whey. The nitrogen content of the shavings was raised from 0.07% to 0.42% (dry matter) when soaked in whey (Table 4).

Table 6 Yield of *Pleurotus sajor-caju* mushrooms (first flush) on water-soaked and whey-soaked shavings (g/100g wet substrate, inclusive of spawn)

Replicate	water-soaked shavings	whey-soaked shavings
1	3.32	contamination
2	3.65	8.43
3	5.08	9.09
4	3.44	contamination
5	2.85	5.49
6	2.42	9.83
7	3.45	10.81
8	4.90	8.46
9	5.09	contamination
10	2.62	contamination
Mean(±S.E)	3.68(±0.32)	8.69(±0.74)

Block *et al.* [1959] determined that thiamine was necessary for the growth of *P. ostreatus* at the rate of 20 µg/L. Hashimoto and Takahashi [1976] found that 100 µg/L of thiamine was the optimum concentration required for mycelial growth and that at higher concentrations there was no significant improvement in growth. Whey powder contains 0.4-0.6 mg of thiamine per 100 g [Walstra and Jenness, 1984]. Since liquid whey is about 6.4% solids, 100 g of liquid whey would contain 25.5-38.3 µg of thiamine. The original shavings were about 60% solids while that of the wet substrate was approximately 30% solids. Assuming that the shavings absorbed their equal weight of whey and that no particular constituent from the whey solids was preferentially absorbed, 100 g of wet substrate would contain 13-

20 μg of thiamine. That level of thiamine would probably be enough to stimulate some growth in *Pleurotus* .

Whether lactose can be easily metabolised by *P. ostreatus* and *P. sajor-caju* still has to be investigated. Oso [1977] studied the effects of different carbon sources on the mycelial growth of *P. tuber-regium*. He found that lactose and galactose provided minimal growth support while glucose and cellobiose were among the best sources. In this experiment, it is suspected that the lactose was used by weed molds rather than by *Pleurotus*, and that the presence of lactose was more of a disadvantage.

The whey-soaked substrate was particularly susceptible to *Trichoderma* infection. In cultivation experiment 1 with *P. ostreatus*, no conclusive observation could be made because of the high incidence of contamination by *Trichoderma* (Table 7).

Table 7 Yield of *Pleurotus ostreatus* mushrooms (first and second flush) from water-soaked and whey-soaked shavings (g/100g wet substrate, inclusive of spawn)

Strain	Block	Water-soaked pasteurised	Water-soaked unpasteurised	Whey-soaked pasteurised	Whey-soaked unpasteurised
P_2	1	4.21	6.17	6.11*	4.92*
	2	5.08	4.91	1.17*	d
	3	4.10	4.88	5.73*	d
	4	5.13	6.58	12.02*	d
Sp_2	1	3.95	6.88	d	3.29*
	2	7.83	6.54	2.68*	d
	3	7.71	d	7.96	d
	4	1.72	d	8.22	d

* contaminated with weed molds
d discarded due to heavy contamination.

Trichoderma viride has been reported to be a dangerous competitor of *Pleurotus* spp. because of their similar nutritional requirements. Also because *T. viride* prefers a more acid medium of pH 4 to 6 [Laborde, 1974], the lower pH of the whey-soaked substrate (pH 4.7 to 4.9) probably increased the susceptibility of the substrate to infection. However, the low pH was not the only predisposing factor to infection since it was found in preliminary tests that there was still a high incidence of infection even if the whey had been neutralised. Underpasteurisation or overpasteurisation will both increase the susceptibility of a substrate to mold infection. In the former case, there would still be pathogenic organisms present to compete for the substrate while in the latter case, the destruction of the thermophilic bacteria would make it easier for the molds to contaminate the substrate. The proper pasteurisation regime depends on several factors such as the physical and chemical properties of the substrate, and the nature of the supplementation. Pasteurisation treatments used for *Pleurotus* cultivation so far have been based on and adjusted from previous reported practices, which may not be necessarily the best. A more systematic method to determine the optimum pasteurisation treatment is therefore required. Laborde *et al.*, [1984] recommended maintaining the temperature of the substrate to 60-61°C for 12-18 hours while supplying a constant supply of fresh filtered air to avoid anaerobic conditions. A conditioning treatment, which consists of exposing the substrate to a lower temperature after pasteurisation, has been found to reduce the risk of infection [Visscher, 1984; Chilton, 1984]. In cultivation experiment 2, the substrate was subjected to a conditioning treatment of 45°C for 48 hours after pasteurisation at 70°C for 18 hours. Although the incidence of infection by *Trichoderma* was reduced (Table 6), the problem was still not completely solved. A chemical treatment in combination with pasteurisation might have been better in this case to protect the substrate from infection.

2.2.4.3 Effect of soya flour and canola meal supplementation on yield

Supplementation of Aspen shavings with soya flour or canola meal improved the yield of *P. sajor-caju* mushrooms. With 3% level (wet weight basis) of supplementation, the yields were 8.64% and 7.94% (from first flush) for the soya flour and the canola meal respectively (Table 8). These yields are more than twice the 3.2% (first flush) obtained for the unsupplemented sterilised substrate (Table 5). There was no significant difference ($p \leq 0.05$) in yield using either source of supplementation.

Table 8 Yield of *Pleurotus sajor-caju* mushrooms (first flush) on Aspen shavings supplemented with 3% canola meal or soya flour (g/100g wet substrate, inclusive of spawn)

Replicate	canola meal	soya flour
1	6.37	9.34
2	7.58	8.01
3	8.67	8.67
4	9.01	8.04
5	8.63	8.58
6	8.51	9.08
7	6.83	8.74
Mean (±S.E)	7.94(±0.39)	8.64(±0.19)

Canola meal has a lower protein content but a slightly higher level of fat than the soya flour (Table 9). It is cheaper than soya flour and is a waste product of the canola oil industry [Clandinin, 1981]. Its use as a source of supplementation for the cultivation of Oyster mushrooms would therefore be preferred.

Table 9 Proximate composition of canola meal and soya flour (%)

	Canola meal*	Soya flour**
Moisture	7	7
Protein	38	52
Fat	4	1
Crude fiber	11	3

*Data from Clandinin [1981]
**Data from Archer Daniels Midland Co.

2.2.4.4 Effects of different levels of canola meal supplementation on yield of *Pleurotus sajor-caju* mushrooms

Mycelial growth and yield of *Pleurotus sajor-caju* were increased with 3% and 5% canola meal supplementation but at the 7% and 10% level, there was no growth (Plate 3) and a pungent smell was noticeable from the substrate. The yield of mushrooms was the highest at the 5% level of supplementation (Plate 4) with a value of 22.14% (g fresh mushrooms/100 g wet substrate) from the two flushes (Table 10). Visscher [1984] reported that the yield being obtained for commercial *Pleurotus* production is about 10-15% of the wet weight of substrate. A yield of 20-25% (wet substrate basis) is considered satisfactory for commercial production [Nagy, personal communication, 1987]. The yield obtained from Aspen shavings with the 5% canola meal supplementation, therefore, indicates that this substrate has good potential for commercial application.

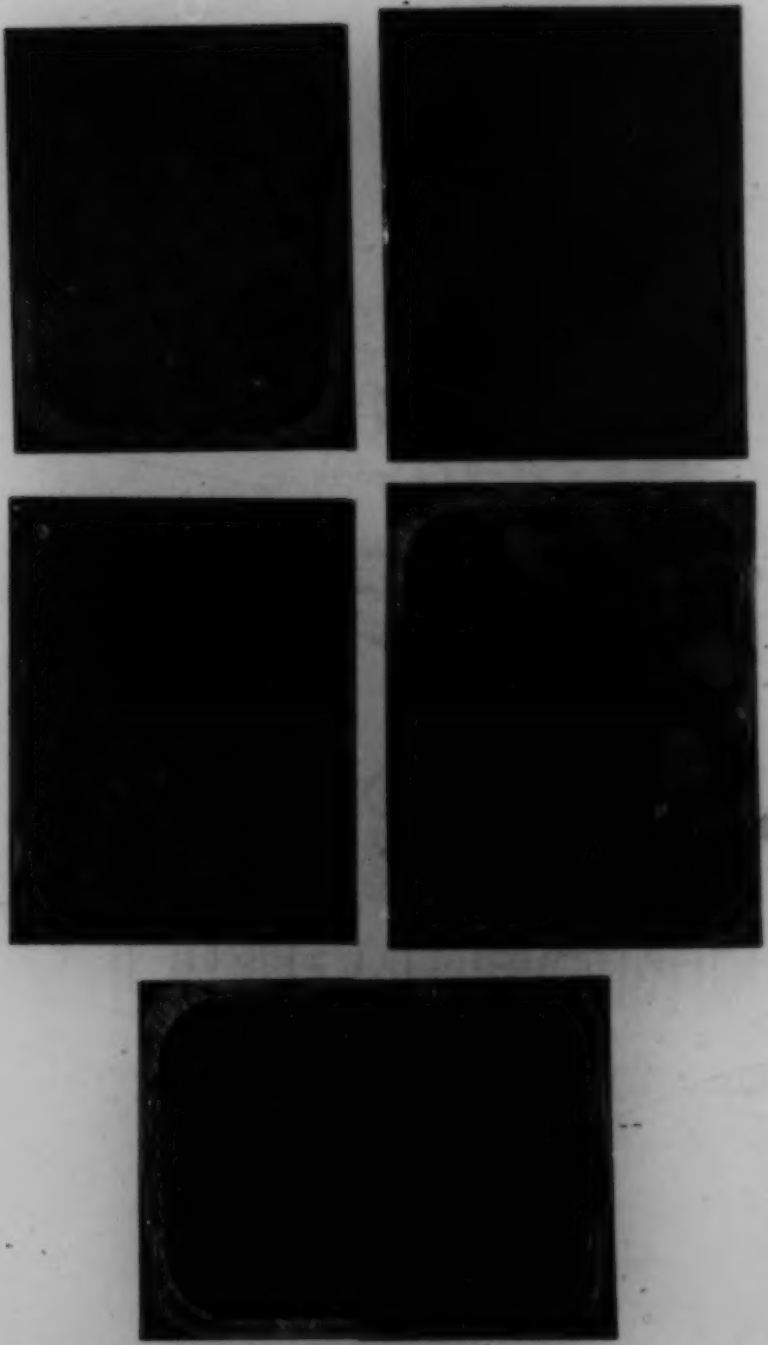


Plate 3 Growth of *P. sajor- caju* on Aspen shavings with canola meal at different levels of supplementation.
A-0%, B-3%, C-5%, D-7%, E-10%

Plate 4 Growth of *P. sajor-caju* mushrooms on Aspen shavings with canola meal at different levels of supplementation. A-0%, B-3%, C-5%

Table 10 Yield of *Pleurotus sajor-caju* mushrooms from Aspen shavings supplemented with canola meal (g/100g wet substrate, inclusive of spawn)

Replicate	Level of canola meal supplementation*		
	0%	3%	5%
1	3.91	11.69	13.21
2	3.28	10.83	12.26
3	2.40	10.06	13.31
4	2.89	8.46	15.21
5	2.49	11.77	16.01
Mean (±S.E) **(First flush)**	2.99(±0.28)	10.56(±0.61)	13.96(±0.70)
1	1.37	6.83	7.03
2	1.80	5.13	8.05
3	1.94	6.61	9.57
4	2.42	5.41	6.67
5	1.77	6.14	9.40
Mean (±S.E) **(Second flush)**	1.86(±0.17)	6.03(±0.33)	8.14(±0.59)
Total yield	4.85(±0.23)	16.59(±0.81)	22.149(±0.96)

*At 7% and 10% supplementation, no fruitbody was produced.

One of the factors affecting fungal growth is the C:N ratio [Deacon, 1980]. The increasing yield of *P. sajor-caju* mushrooms with increasing canola meal supplementation at 3% and 5% could have been due to the decreasing C:N ratio of the substrate (Table 11). With a C:N ratio of less than 36:1 as obtained with 7% of canola meal supplementation the mycelium did not grow throughout the substrate. Several causes may be involved but these would have to be confirmed by further studies. This level of nitrogen supplementation could have been inhibitory to the growth of the mycelium. The high nitrogen content could have promoted the growth of bacteria in the substrate to the detriment of *Pleurotus* or there may have been some substances in the canola meal which were toxic to the *Pleurotus* mycelium at these levels of supplementation. The optimum C:N ratio for a particular organism will depend on the characteristics of the substrate and on the source of the supplementation. Jauhri and Sen [1978c], found that the C:N ratio giving the best growth for *P. ostreatus* is 30:1 using as substrate, sugarcane bagasse supplemented with urea. In this study, the C:N ratio which gave the best yield was 47:1 using as substrate aspen shavings and canola meal supplementation.

Table 11 C/N ratio of Aspen shavings, whey-soaked shavings, and shavings supplemented with canola meal

Samples	C:N ratio
Aspen shavings	400:1
Canola meal	7:1
Shavings and 0% canola meal	400:1
Shavings and 3% canola meal	70:1
Shavings and 5% canola meal	47:1
Shavings and 7% canola meal	36:1
Shavings and 10% canola meal	27:1

2.2.4.5 Nitrogen content of fruitbodies

Supplementation of the substrate with whey or with canola meal was found to increase the nitrogen content of the fruitbodies. Mushrooms grown on wood shavings alone had a nitrogen content of about 3.8% (dry weight) while those from the whey-soaked shavings had about 4.3% nitrogen (dry weight). With 3% and 5% canola supplementation, the nitrogen content rose to 3.9% and 4.6% respectively (Table 12). Increase in the nitrogen content of the fruitbodies from substrate supplemented with a source of nitrogen has been reported by several authors. Bano *et al.* [1979] observed that *P. flabellatus* mushrooms harvested from rice straw supplemented with cotton seed powder had a higher protein content than those from the unsupplemented straw. Zadrazil [1980] found that supplementation of wheat straw with 30% soya bean meal increased the nitrogen content of *P. sajor-caju* fruitbodies by more than two-fold compared to the fruitbodies from straw. Whether this increased nitrogen level has any significant effect on the structure and nutritional value of the mushrooms needs to be assesed further. Bano *et al.*, [1979] reported that the *P. flabellatus* mushrooms from the supplemented substrate were more tender in texture (as measured by the Ottawa Texture Measuring System) and appeared to have a more intense flavour. A sensory evaluation would be needed to confirm these observations. The nature of the nitrogen source may modify the amino acid content of the fruitbodies. Significant differences were found in the amino acid content of mushrooms grown from composts supplemented either with ammonium sulphate or a mixture of urea plus ammonium sulphate [Crisan and Sands, 1978].

Table 12 Nitrogen content of *P. sajor-caju* mushrooms.

substrate	% Nitrogen (dry weight)
Shavings with no supplementation	3.76
Shavings and 3% canola meal	3.88
Shavings and 5% canola meal	4.55
Water-soaked shavings	3.79
Whey-soaked shavings	4.28

The nitrogen content also seems to vary according to the part of the fruitbodies. Oddson [1979] showed that the stems of *P. florida* mushrooms had a lower nitrogen content than the caps. A similar observation was made for the nitrogen content of the cap and stem of the sporeless strain of *P. ostreatus* used in this study (Table 13).

Table 13 Nitrogen content of *P. ostreatus* (sporeless strain) mushrooms

Sample	Substrate	% Nitrogen (dry weight)
caps	Water-soaked shavings	3.3
caps	Whey-soaked shavings	5.3
stems	Water-soaked shavings	2.2
stems	Whey-soaked shavings	3.2

2.2.4.6 Normal and Sporeless strains of *P. ostreatus*

There was no significant difference in yield between the normal (P_2) and sporeless (Sp_2) strains of *P. ostreatus* (Table 7). The two strains were, however, very different in appearance (Plate 5). The fruitbody of the normal strain had a fan-shaped, gray brown cap (2.5-10 cm) with a short stipe (1-2 cm) which was in an adaxial position. The cap of the sporeless strain was circular with a dent in the middle, gray brown and 2.5-9 cm in diameter; the stipe was relatively long, thick, central, and 4-10 cm long. The cap to stem ratio of the normal strain was about six times that of the sporeless strain (Table 14).

Table 14 Ratio of cap to stem for *P. ostreatus* mushrooms grown on water-soaked shavings

No of samples	Strain	Ratio (cap:stem)
10	Normal	9.0:1
8	Sporeless	1.4:1

The stem of the sporeless strain was much tougher in texture than the cap (Table 15) and may be therefore undesirable from the consumer point of view. Scanning electron microscopy of the gill surface of the Sp_2 fruitbody showed that the basidia did not bear any spore (Plate 6). Although the sporelessness of this strain would solve the allergy problems associated with the harvest of mature *Pleurotus* fruitbodies, its morphological characteristics make it unsuitable for commercial purposes. There are other sporeless strains on the market and it would be worthwhile to investigate their suitability.

Table 15 Shear values of *Pleurotus ostreatus* mushrooms grown on water-soaked shavings (determined by the Ottawa Texture Measuring System)

Strain	Mushroom part	No. of samples	Shear value (kg) Mean (±S.E)
Normal	caps	11	90 (±4)
Sporeless	caps	7	70 (±3)
	stems	4	186 (±16)

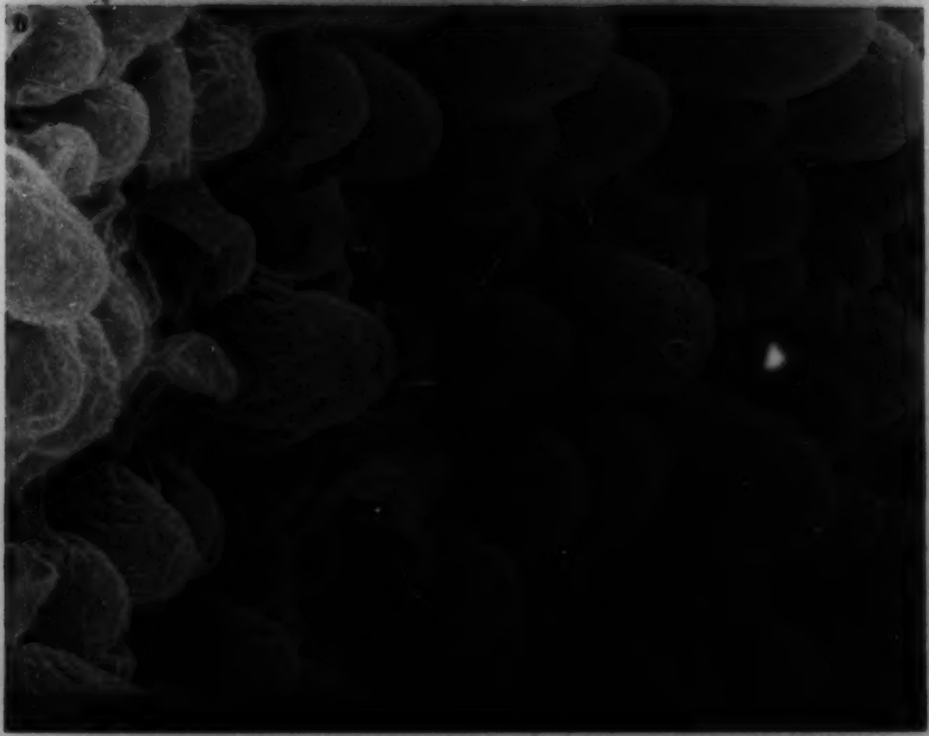
2.2.5 REMARKS

Pleurotus sajor-caju grows faster and produces fruitbodies more rapidly than *P. ostreatus*. The time taken for a cultivation run from the time of inoculation to the time of harvest of the fruitbodies from the first flush was 45-48 days for the strains of *P. ostreatus* studied and 20-22 days for *P. sajor-caju*. Also, *P. ostreatus* required a drastic temperature shock (from 25°C to less than 15°C) for the induction of fruitbodies, which took about 10 days while for *P. sajor-caju*, the temperature was lowered only by 3-4°C and the primordia appeared within 2-3 days. For the commercial grower, *P. sajor-caju* would be a better species to exploit since a higher yield can be obtained within a period of time.

Aspen shavings substrate with 5% canola meal supplementation was found to be suitable for the growth of *P. sajor-caju* giving a yield of about 22% (wet substrate basis) from two flushes. The yield of *P. sajor-caju* mushrooms on whey-soaked shavings was about 8% (wet substrate basis) from the first flush and the substrate was particularly susceptible to *Trichoderma* infection.

Plate 5 Morphology of *Pleurotus ostreatus*.
A-Normal strain (P_2). B-Sporeless strain (Sp_2)

Plate 6 Scanning electron micrographs of gill surface of *Pleurotus ostreatus*. A-Normal strain (P_2). B-Sporeless strain (Sp_2).



CHAPTER 3

DEHYDRATION OF OYSTER MUSHROOMS

3.1 LITERATURE REVIEW

3.1.1 DEHYDRATION OF MUSHROOMS

Two methods of commercial importance for the drying of mushrooms are air-drying and freeze-drying. Air-drying is used for many varieties of wild mushrooms and some cultivated species such as *Lentinus edodes* and *Volvariella volvacea* while freeze-drying is limited to a smaller volume of mushrooms because of the high cost of this process [Cho *et al.*, 1982].

In Western Europe and North America, where the predominantly cultivated mushroom species is *Agaricus bisporus* (the button mushroom), dehydration of mushrooms is limited to the powdered and sliced products that are used primarily in soups and sauces [Oddson, 1979]. Air-dried mushrooms, like other foods dried by air at relatively high temperatures are subject to numerous undesirable changes as listed by Harper and Tappel [1957], including: (1) pronounced shrinkage, (2) migration of solutes to the surface, (3) denaturation of proteins, (4) case-hardening, (5) undesirable reactions in heat-sensitive materials, (6) excessive loss of desirable volatile constituents, and (7) poor rehydratability. Added to these are the problems of browning and the tough texture of the products. Freeze-dried products, on the other hand, are not shrunken, have good flavour and rehydrate fast. However the process is expensive because of the freezing stage and the need for a high vacuum during drying. Problems concerned with the handling and treatment of the raw materials prior to the drying process are usually the same as those encountered for other processes, such as canning and freezing. One example is the problem of enzymatic browning when the mushrooms are cut and sliced.

3.1.2 BROWNING IN MUSHROOMS

Browning is a phenomenon observed in many food processing operations whenever plant tissues are cut or bruised. Onslow [1921] reported on the role of oxidative enzymes as the primary cause of this reaction. Now collectively known as phenolase or polyphenoloxidase (PPO), these enzymes were known under several other names such as tyrosinase by early investigators. Malette and Dawson [1949] reported the isolation of several highly purified tyrosinase preparations from the mushroom *Psalliota campestris*. Each tyrosinase form possesed a particular catalytic activity and had the ability to catalyse the aerobic oxidation of both monohydric and o-dihydric phenols. Phenolases are found in almost all plant tissues and have the ability to oxidise phenolic compounds to o-quinones [DeMan, 1980] which then polymerise nonenzymatically to form dark coloured melanins [Kahn, 1985]. The level of phenolase present in the plant tissues may, however, vary from species to species. Oddson [1979] found that there was lower phenolase activity and substrate concentration in *Pleurotus florida* compared to *Agaricus bisporus*. Although the phenolases have their biological importance in plants [Schwimmer, 1981], their activities are undesirable to the food processor and have to be controlled or inhibited.

Browning can also be caused by reactions not catalysed by any enzyme. Non enzymatic browning (Maillard reactions) results from the interaction of the carbonyl group of a reducing sugar and the amino group of an amino acid or protein to form brown polymeric insoluble substances [King, 1975]. The rate of the reactions depends on several factors such as concentration of reactants, pH and temperature [DeMan, 1980].

3.1.3 CONTROL OF ENZYMATIC BROWNING BY BLANCHING

Blanching consists of either dipping the food material in water at 70-105^{o}C or exposing the product to steam in order to destroy the enzymes by denaturing the proteins [Schwimmer, 1981]. Cruess and Mrak [1942] found that steam-blanching of mushrooms for 5-10 mins prior to drying resulted in a product of superior flavour but of less attractive appearance than unblanched mushrooms. Brunell *et al.* [1943] reported a similar finding concerning the flavour of blanched mushrooms and observed that the unblanched mushrooms were very dark in colour. In contrast, Komanowsky *et al.* [1970], and Oddson and Jelen [1981] observed that unblanched air-dried mushrooms had good flavour and storage stability and a better colour and shape than the steam-blanched dried samples. Fang *et al.* [1971] found that blanching mushrooms in boiling water for 2 minutes prior to freeze-drying resulted in a product with better colour and rehydratability but of inferior flavour and aroma. They also noted a loss of soluble solids (amino acids, sugars, minerals, and flavour components) to the water. Blanching leads to shrinkage and this may affect the final texture of the dried products. Yeh [Oddson, 1979] reported that blanching had the effects of increasing the toughness of both the rehydrated air-dried and freeze-dried mushrooms.

3.1.4 USE OF SULFITES FOR BROWNING CONTROL

A method employed by industry to control browning in foods is by the use of additives. Embs and Markakis [1965] reported the inhibition of mushroom tyrosinase and presented evidence that sulfite prevented browning by combining with enzymatically produced 0-quinones thereby stopping their condensation to melanins. Luh and Eidels [1969] observed that freeze-dried mushrooms pretreated with 1% sodium bisulfite had substantially better colour on rehydration.

Komanowsky [1970] also found that sodium bisulfite decreased mushroom discoloration during drying but recommended that the level be kept below 100 ppm as higher concentrations would result in an aftertaste that may be objectionable. Fang *et al.* [1971, 1976] found that mushrooms dipped in 200 ppm of SO_2 solution prior to freeze drying retained a better colour and that a 0.1% sodium metabisulfite strongly inhibited phenolase activity. The effect of SO_2 and metabisulfite can also be attributed to their capacity to reduce quinones back to their parent o-diphenols [Walker, 1977]. Instead of dipping the mushrooms in sufite solution, they could alternatively be exposed to SO_2 gas. Hale and Tressler [1970] reported that mushrooms can be preserved by treating them at ambient temperature with gas containing 9.25% by volume SO_2 for 10 seconds to 30 minutes, packaging and then freezing. In combination with other chemicals the inhibitory effect of SO_2 might be enhanced. Voinovitch *et al.* [1949] found that thiamine, or nicotinic acid, in combination with SO_2 was superior to SO_2 alone with respect to enzyme inactivation. Hanson [1975] recommended dipping washed mushrooms in an aqueous solution of 400 ppm of SO_2 with 6% sodium chloride and 0.5% citric acid to prevent discoloration of the raw material. Sulfites are also effective in controlling non-enzymatic browning [De Man, 1980]. It is believed that sulphur dioxide reacts with the degradation products of the amino sugars and thereby prevents the condensation of these compounds into the melanoidin pigments.

Recently, there has been some controversy concerning the potential hazards of sulfites to consumers. Sulfites were reported to be the causative agents for initiating asthmatic reactions in a small subset of the asthmatic population, and for having mutagenic effects at very high doses [Taylor and Bush, 1986]. Sulfites are now banned in fresh fruit (except in grapes) and vegetables that are intended for raw consumption but are still allowed in processed foods, in which case sulfite labelling is required on the packages [Food and Drugs Act and Regulations, 1987, Section B 11.001.1].

3.1.5 OTHER CHEMICALS

Several other chemicals have also been studied for their inhibitory effects on browning in mushrooms. Brunell *et al.* [1943] reported that mushrooms dipped for 5 minutes in 2 and 3 percent citric acid were almost white in colour after drying. However, Komanowsky *et al.* [1970] observed that citric acid had no effect on the colour of the air-dried mushrooms. McCord and Kilara [1983] found that citric acid at pH 3.5 inactivated phenolase activity and observed the inhibition of non enzymatic reactions. Ascorbic acid is a reducing agent and like SO_2 can prevent enzymatic browning by reducing the colourless o-quinones back to their parent phenols. However, once all the ascorbic acid has been consumed, the o-quinone is no longer reduced and can undergo oxidative polymerisation to the brown pigment [Walker, 1977]. Komanowsky *et al.* [1970] noted little effect on the colour of air-dried mushrooms pretreated with 1% ascorbic acid. Fang *et al.* [1976] came to a similar conclusion when they investigated the inhibiting effect of 0.25% ascorbic acid on the activity of polyphenoloxidase from frozen mushrooms. A combination of ascorbic and citric acid may be more effective since the citric acid will provide an acid environment which is stable for the ascorbic acid [Furia, 1977].

Amino-acids, peptides, and proteins are believed to control enzymic browning in at least two ways: by reacting with o-quinones, the products formed by PPO activity; and by chelating the essential copper at the active site of PPO. L-lysine, L-histidine, L-phenylalanine and L-cysteine were found to inhibit o-dihydroxyphenolase activity of mushroom PPO, with L-cysteine being the most effective. Glycine, diglycine, and triglycine decreased the final level of melanin

[Kahn, 1985]. Since amino acids are non-toxic, they could be safely added to food during processing to control browning. However, as a pretreatment for drying mushrooms, these additives may not be desirable since they could enhance non-enzymatic browning.

3.1.6 CONTROL OF BROWNING BY CARBON MONOXIDE

Exposing the mushrooms to carbon monoxide has been found by some researchers to be effective in preventing browning in mushrooms. Fujimoto *et al.* [1972] aerated Shiitake mushrooms packed in a dessicator with carbon monoxide for two hours and fifty minutes and found that browning was prevented while there was no effect on flavour. Robitaille and Badenhop [1981] studied the effects of hyperbaric storage (at 35 atm.) on the button mushrooms. They found that at a partial pressure of 0.035 atm., carbon monoxide reduced browning but not respiration. When the carbon monoxide level was returned to 0.001 atm. the mushrooms suffered no injury due to decompression. The inhibitory activity of carbon monoxide may involve the active site copper in the phenolase enzyme [Schwimmer, 1981].

3.1.7 TEMPERATURES USED FOR AIR-DRYING

One of the main factors determining the final quality of the dried product is the temperature being used in the process. Cruess and Mrak [1942] recommended that the mushrooms should be dried at a temperature not exceeding 66°C (150° F). Brunell *et al.* [1943] found that a temperature within a range of 63-66°C (145-150° F) was suitable. A method of air-drying mushrooms which resulted in products with comparable flavour to the freeze-dried mushrooms has been reported [Anon, 1968a]. In this process, mushrooms were first treated with 500 ppm of chlorine solution for 10 minutes to reduce the microbial population and with sulfite to prevent browning. The mushroom pieces were then dried in a two step process: at 43^oC for 3 hours followed by 1 hour at 79^oC. The products apparently still maintained their quality after seven months of storage. Komanowsky *et al.* [1970] found that a dip in chlorine water (800 ppm) for 5 to 10 minutes did not adequately reduce the microorganisms normally present in the mushrooms. They reported that by combining a low temperature drying of 38-43^oC (100-110^o F) in the first stage with high temperature drying 77-82^oC (170-180^oF) in the second finishing stage, the products obtained were light in colour and with relatively low number of organisms.

During air-drying, two main periods can be recognized: (1) a constant-rate period, during which the rate of water removal is essentially steady because the surface of the material is kept wet, (ii) a falling-rate period, during which the water supply within the food is inadequate to keep the surface saturated; this is due to the increasing difficulty of moisture to move from the capillaries and interstices of the material to the surface [Considine, 1982]. During the constant-rate period, the temperature of the surface is approximately that of the wet-bulb temperature due to evaporative cooling. This means that initially the food material is protected from the damaging effects of a higher temperature. During the falling rate period, the temperature of the food material begins to rise eventually reaching that of the surrounding air [Van Arsdel, 1973]. As the moisture content of the food decreases, however, the food is more tolerant to the effects of heat since the thermal conductivities of dry foods are low [Karel, 1975]. Too high a temperature at the beginning of the drying process will produce a layer of closely packed shrunken cells sealed together at the surface hindering further moisture removal [Geankoplis, 1978]. This will also enhance the activity of enzymatic and non-enzymatic browning. Thus a two stage drying process involving an initial lower temperature followed by a higher temperature is recommended by several researchers [Anon, 1968b; Komanowsky *et al.*, 1970; Pruthi *et al.*, 1978].

However, the use of a single appropriate temperature can still give suitable products.

For the air dehydration of the Oyster mushrooms, *Pleurotus ostreatus*, Gormley and O'Riordain [1976] recommended a temperature of 51-65° C for 5-6 hours. Oddson [1979] dried *Pleurotus florida* mushrooms at 65° C for 3 hours in a cabinet drier but observed the development of a brownish-yellow colour. One method reported by Cho *et al.* [1982] for the air drying of Shiitake consists of dehydrating the mushrooms first at 30° C and increasing the temperature by 1-2° C per hour to 50° C in 12-13 hours. The products are finally heated to 60° C for one hour to enhance the flavour and bring out the lustre of the cap. *Volvariella volvacea*, the straw mushroom is dried at an initial temperature of 40° C which is gradually increased to 45° C for a period of eight hours [Cho *et al.*, 1982]. Pruthi *et al.* [1978] recommended for the dehydration of the straw mushroom, a temperature of 70° C for two hours, followed by drying at 65°C for two hours and for another four hours at 55-60° C.

3.1.8 FREEZE-DRYING OF MUSHROOMS

Freeze-drying consists of freezing the material and then subliming the water from the solid state (ice) to the gaseous state (water vapor) under conditions below the triple point of water [Karel, 1975]. Keeping the material frozen until it is dry minimises shrinkage and migration of dissolved materials and prevents chemical reactions [Harper and Tappel, 1957]. The reduced shrinkage results in a porous product which facilitates the escape of water vapor during drying and allows a rapid and nearly complete rehydration. Although considered so far to be the best dehydration method for many foods, there are still many problems associated with the freeze-drying process which result mainly from damage in the raw materials during the freezing stage [King 1975]. Rapid freezing is reported to cause less structural damage because of the formation of smaller ice crystals [Fennema, 1975]. However rapid freezing may result in a greater loss of volatile components. Bartholomai *et al.* [1975] reported a significant reduction in the retention of a natural volatile in liquid mushroom extract frozen rapidly in liquid nitrogen compared to the extract frozen slowly in still air at -40^{o}C. Flink [1975] suggested that slow freezing allows a greater incorporation of volatiles into the solute phase and favours the formation of microregion structures in which the volatiles are entrapped and preserved. Thus rapid freezing may minimize structural damage but it does not maximize volatile retention.

Bartholomai [1974] reported obtaining products of acceptable quality by a combination of freeze-drying at 60^{o} C and air-drying at 80^{o} C. By this process, the drying time could be cut by 28% compared to the time required for freeze-drying. The combination of freeze-drying and air-drying processes was patented by Kruger in1969 [Hanson, 1975]. The initial freeze-drying stage is said to ensure that the external layer of the food becomes porous so that movement of moisture from within the food during air-drying is facilitated.

3.1.9 ALTERNATIVE METHODS OF DRYING MUSHROOMS

Sun drying is a low cost method of drying used for wild as well as cultivated mushrooms such as the Wood-ear fungus (*Auricularia*) and Shiitake. Oddson and Jelen [1981] found that Oyster mushroom (*Pleurotus florida*) caps, could be satisfactorily sun-dried without being first blanched. The products were reported to be not substantially different from hot air-dried material. Sun-dried products, however, may be more susceptible to spoilage by moulds. Cho *et al.* [1982] reported that some countries such as Australia ban the importation of sun-dried mushrooms unless they have been heat treated to 60° C for four hours. Air-dried and freeze-dried mushrooms may also carry a relatively high bacterial count [Li *et al.*, 1974]. These authors found that the count could be reduced to two thirds or less after a 2,450 Mhz microwave irradiation for 30 to 90 seconds. The moisture content of the mushroom slices was also reduced from 9% to 6% during the process. Wagner *et al.* [1980] recommended a pretreatment by sodium hypochlorite and sodium bisulphite to reduce the bacterial count in air-dried mushrooms while a combination of sorbic acid and bisulphite was more effective for sun-dried products.

Wang *et al.* [1975] reported that a combination of vacuum-drying and microwave drying produced dehydrated mushroom slices which were close in quality to freeze-dried products. The method consisted of drying the mushroom slices at 25°C under a vacuum of 30 mm Hg for two hours; the temperature was then increased to 40°C for another 2.5 hours. The final stage drying was carried out either by microwave (3 minutes with 4.8 kW transmitted power) or by tunnel drying (65°C for 1.5 hours). Drum drying has also been investigated for dehydration of mushrooms. Komanowsky *et al.* [1966] reported the production of a dried mushroom powder by drum drying; they claimed that this product was superior to a freeze dried mushroom powder.

3.1.10 MOISTURE CONTENT OF DRIED MUSHROOMS

The basic objective in drying food products is the removal of water from solids to a moisture level at which microbial spoilage is minimized [Fortes and Okos, 1980]. On the other hand, the storage stability of a dehydrated food is enhanced by leaving a trace of moisture, equivalent to a monolayer of water, to "coat" all internal and external surfaces. This monolayer is believed to serve as a barrier between atmospheric oxygen and reactive substances in the food [Salwin, 1959; Labuza *et al.*, 1970]. Brunnel *et al.* [1943] recommended dehydrating mushroom slices to a moisture content of 5% or below. Komanowsky *et al.* [1970] advised a range between 4% and 8%. They found that mushrooms dried to a moisture content below 4% were tough on reconstitution and poor in flavour. Above 10% moisture content, colour and flavour changes occured readily. Fang *et al.* [1971] freeze-dried the mushrooms to 2.53-3.54% in their study of the effects of additives to prevent browning. Gormley and O'Riordain [1976] found that a moisture content of about 6% was associated with an unacceptable yellow gill colour in the dehydrated *Pleurotus ostreatus* mushrooms. They recommended drying the mushrooms to a level of 1%. However, this value seems very low and at this level, the possibility exists that there may be some oxidation reactions between oxygen and exposed active sites of the dehydrated foods. Funfalek [1980] in his guidelines for drying edible fungi recommended dehydrating the mushrooms to a moisture level of 12%. Cho *et al.* [1982] advised that the moisture content of dehydrated Shiitake mushrooms should not exceed 13% to avoid microbial spoilage. Pruthi *et al.* [1978] carried out sorption isotherms studies on dehydrated powder (6.5% moisture) of *Volvariella volvacea* mushrooms. They found that the equilibrium relative humidity (ERH) of the sample was 52%, at which level the powder neither picked up nor lost any moisture. The sample retained its quality up to 8% moisture while at 9% and above, it became moist and unacceptable. When the dried mushrooms were stored in air tight friction-top tins (wax-sealed) at room temperature, the quality was maintained up to 1 year, after which slight yellowing was observed.

Based on the information reviewed here, it seems that dehydrating the mushrooms to a moisture content of 5-8% would be suitable. Once dehydrated, the products should be packaged in air-tight containers, preferably under nitrogen to avoid oxidation and moisture absorption.

3.1.11 FOOD VALUE OF DRIED MUSHROOMS

Mushrooms are primarily valued for their unique flavour properties. Therefore, the main concern for the food processor would be to maximize the retention of the flavour components in the dried products. Dijkstra and Wiken [1976] studied the constituents contributing to mushroom flavour in the button mushroom, *Agaricus bisporus*. They found that nucleotides, amino-acids and carbohydrates played a significant role. Among the volatiles, 1-octen-3-ol was the most important compound. The nucleotides, GMP (Guanosine monophpsphate), and AMP (Adenosine monophosphate) acted by enhancing the flavour of glutamic acid. Dijkstra [1976] later compared the content of 3 flavour compounds in fresh and air-dried button mushrooms. His results indicated that there was a 76% reduction of 1-octen-3-ol, 68% in 5'GMP, and 17% in L-Glutamic acid. Sulkowska and Kaminski [Maga, 1981] found an even greater loss of about 90% 1-octen-3-ol in dried button mushrooms. Dijkstra [1976] found that fresh *P. ostreatus* contained five times more 1-octen-3-ol than fresh *A. bisporus*. So far, the effects of processing have been focused on what are believed to be the main flavour components such as 1-octen-3-ol. However, the flavour chemistry of mushrooms is complex and several classes of compounds are involved. Furthermore, these compounds may vary drastically among species [Maga, 1981]. Although air-drying may lead to a significant loss in flavour compounds, the drying process itself may impart a desirable flavour to the mushrooms. Air-dried Shiitake has a special aroma not present in the fresh msuhrooms [Cho *et al.*, 1982].

Very few studies have been carried out on the nutritive value of dehydrated mushrooms. Nutrients of importance that would be affected by heat are mainly the B vitamins. Brunell *et al.* [1943] found that there was a loss of 18% in thiamine and 12% in nicotinic acid in button mushrooms that had been blanched and dehydrated at temperatures between 63-66^{o}C (145-150^{o} F) while the loss of riboflavin was very low. Thiamine is heat sensitive while niacin and riboflavin are fairly stable to heat [Fleck, 1981]. In addition, thiamine can be destroyed by sulphur dioxide.

3.2 DRYING EXPERIMENTS

3.2.1 OBJECTIVES

The aim of this study was to compare three drying methods: air-drying, freeze-drying and high vacuum-drying for their effects on the microstructure and rehydration properties of the Phoenix Oyster mushroom, *Pleurotus sajor-caju*. The rehydrated mushrooms were evaluated for sensory characteristics such as texture, flavour and overall acceptability. The Phoenix Oyster mushroom was chosen in this study because of its rising importance in the exotic mushroom industry in Canada. However, the general objective was to find a suitable method of drying that can be applied to high-value mushrooms such as the Chanterelle, *Cantharellus cibarius*.

3.2.2 MATERIALS AND METHODS

3.2.2.1 Mushrooms

Fresh *Pleurotus sajor-caju* mushrooms (Strain P60, Hauser, Champignonkulturen, Switzerland) were obtained from Mountain Mushrooms Research farm (Airdrie, Alberta). Fruitbodies of 7-12 cm diameter were selected. The stems were cut off from the base of the caps. Only the caps were used.

3.2.2.2 Drying treatments

Gormley and O'Riordain [1976] recommended a temperature of 51-65°C for air-drying of *Pleurotus ostreatus*. Bartholomai [1974] reported obtaining products of satisfactory sensory quality from mushrooms dried by a combination of freeze-drying at 60°C and air-drying at 80°C. A temperature of 55°C was used in this work for all three drying methods as a compromise and uniform treatment.

Nine hundred grams of whole mushroom caps on a perforated tray were dried to a moisture content of about 7% by the methods listed below. Each treatment was replicated three times using three different batches of mushrooms. The methods were:

1 Air drying in a forced convection oven (model LDB-69, Despatch Industries Inc. Minneapolis) for 10 hours.

2 Freeze drying for 20 hours. Mushrooms were previously frozen in a walk-in freezer (air-blast) at -30°C. They were next dried in a RePP freeze drier (Virtis Co. Inc., Gardiner, N.Y.); the shelf temperature was maintained at 55°C and the condenser temperature at -60°C; the vacuum was less than 1 mm Hg.

3 High-vacuum drying, using the equipment and drying conditions as in 2, but omitting the freezing step.

3.2.2.3 Packaging

Dried mushrooms were stored in moisture-proof storage bags at room temperature until they were evaluated for rehydration and textural properties after a period of 2 months.

3.2.2.4 Temperature changes in mushrooms during vacuum drying

Several thermocouples were placed in a 10 cm diameter mushroom cap and the temperature changes were monitored during vacuum drying. Additional thermocouples were used to record temperatures of the environment in the dryer.

3.2.2.5 Scanning electron microscopy (SEM)

Samples were prepared according to the method described by Nickerson *et al.* [1974] for fungal spores. The samples were prepared within a week after the drying treatments. For the observation of the gill surface, small pieces of approximately 5 mm^2 were cut out about halfway between the stipe and the edge of the pileus. For the hyphae, samples of similar size were cut out from the inner tissue of the cap close to the attachment of the stipe. Samples were exposed to osmium tetroxide vapour overnight and air dried at room temperature. The dried samples were then sputter coated with gold and examined with the scanning electron microscope (Cambridge streoscan model S250, Cambridge Industries, England) at 20 kV.

3.2.2.6 Moisture content of dried mushrooms

Approximately 3 g samples of dried mushrooms, crushed into small pieces, were placed in an oven at 105°C for 3 days. Moisture loss was then calculated. Determinations were carried out in duplicate.

3.2.2.7 Rehydration time

About 2 g of dried mushrooms were soaked in 500 mL of water. The rehydration experiments were carried out at two different temperatures of 20°C and 90°C. After a specific time interval, the mushrooms were removed and excess water gently blotted out by paper towel. The samples were weighed and then placed back into the water. The time interval used for repeated determinations of air-dried mushrooms was 10 minutes while for the freeze-dried and high-vacuum dried mushrooms, it was 30 seconds. Readings were taken until there was no further change in weight, at which time the mushrooms were considered to have reached full rehydration capacity. There were 3 batches and determinations were made in triplicate for each sample from each batch.

3.2.2.8 Rehydration capacity

Rehydration capacity was calculated as the maximum amount of water absorbed (g) by the dry material (g) at the end of the rehydration time experiment. Determinations were made in triplicate for each sample from each of the 3 batches. The rehydration capacity was calculated as follows:

$$\text{Rehydration capacity} = \frac{\text{Maximum amount of water absorbed (g)}}{\text{Amount of dried product (g)}}$$

3.2.2.9 Texture measurements

The textural characteristics of the rehydrated mushrooms were measured by the Ottawa Texture Measuring System (Canners Machinery Ltd. Simcoe, Ontario) developed by Voisey [1971]. Mushrooms were cut into pieces of similar size and samples of 25 g were placed in a 20 cm^2 wire food cell. The force (kg) required by the Kramer shear press to shear and extrude the mushrooms through the slotted cell was used as an indicator of the product's degree of toughness or tenderness. The deformation rate of the press was 12.5 cm/min. Six determinations were carried out for each sample of dried mushrooms from each of 3 batches.

3.2.2.10 Sensory evaluation

Mushrooms were evaluated for appearance, texture, flavour and overall acceptability. The attributes were assessed on a hedonic scale as described by Larmond [1977]. The scores were from 1 for "dislike extremely" to 9 for "like extremely". There was a total of 26 panelists. Fresh, air-dried, freeze-dried and vacuum-dried mushrooms were tested. The dried samples were rehydrated and excess water gently blotted out on a paper towel. Mushrooms were fried in butter for about 2 minutes on each side on medium to high heat in a frying pan. They were lightly sprinkled with salt in order to enhance the flavour. Each sample was given a randomly chosen three digit code. The order of the four samples was randomised for each panelist and the order in which each panelist should taste the samples was indicated. The sensory evaluation was carried out in duplicate by each panelist. Scores were compiled and analysed by the Analysis of Variance (ANOVA). Attribute scores which gave a significant F ratio for sample effect were further analysed by the Tukey's test to determine which samples were significantly different.

3.2.3 RESULTS AND DISCUSSION

3.2.3.1 Structural changes in *P. sajor-caju*

The freeze-dried and vacuum-dried mushrooms were similar in several aspects. They both exhibited good colour, with little or no shrinkage. In contrast, the air-dried mushrooms were darkened and shrunken (Plate 7). At the microstructural level, the hyphae of the air-dried samples were more flattened and collapsed than the freeze-dried and vacuum-dried samples (Plate 8). The basidia of all three samples (Plate 9) were distorted to some extent but there seemed to be less damage on the gill surface of the vacuum-dried samples. The rehydrated samples were difficult to analyse because of the loss of a more discernible configuration once water was absorbed. The rehydrated hyphae of the freeze-dried and vacuum-dried (Plate 10) samples were similar but those of the air-dried samples were more folded. The rehydrated gill surface of the vacuum-dried samples was smooth compared to the freeze-dried samples while the surface of the air-dried samples was considerably wrinkled (Plate 11).

According to Van Arsdel [1973], during drying the solid structural elements of the plant tissues are pulled closer together under the influence of surface tension as water evaporates from the wet surface. This effect eventually spreads

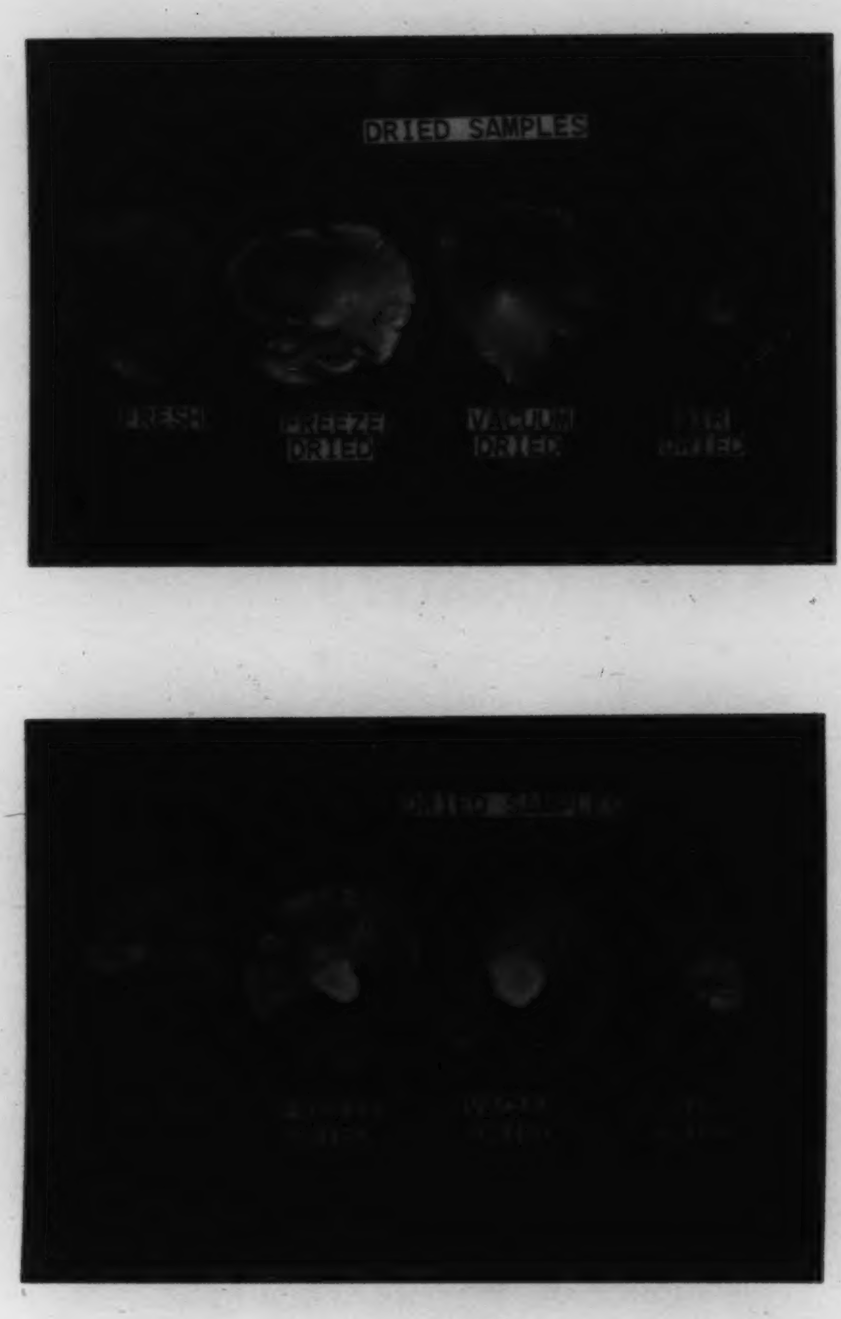

DRIED SAMPLES
FRESH
FREEZE DRIED
VACUUM DRIED
AIR DRIED

Plate 7 Fresh and dried *Pleurotus sajor-caju* mushrooms

Plate 8 Scanning electron micrographs of hyphae from caps of dried
P. sajor-caju mushrooms
A- Air-dried. B- Freeze-dried. C- Vacuum-dried.

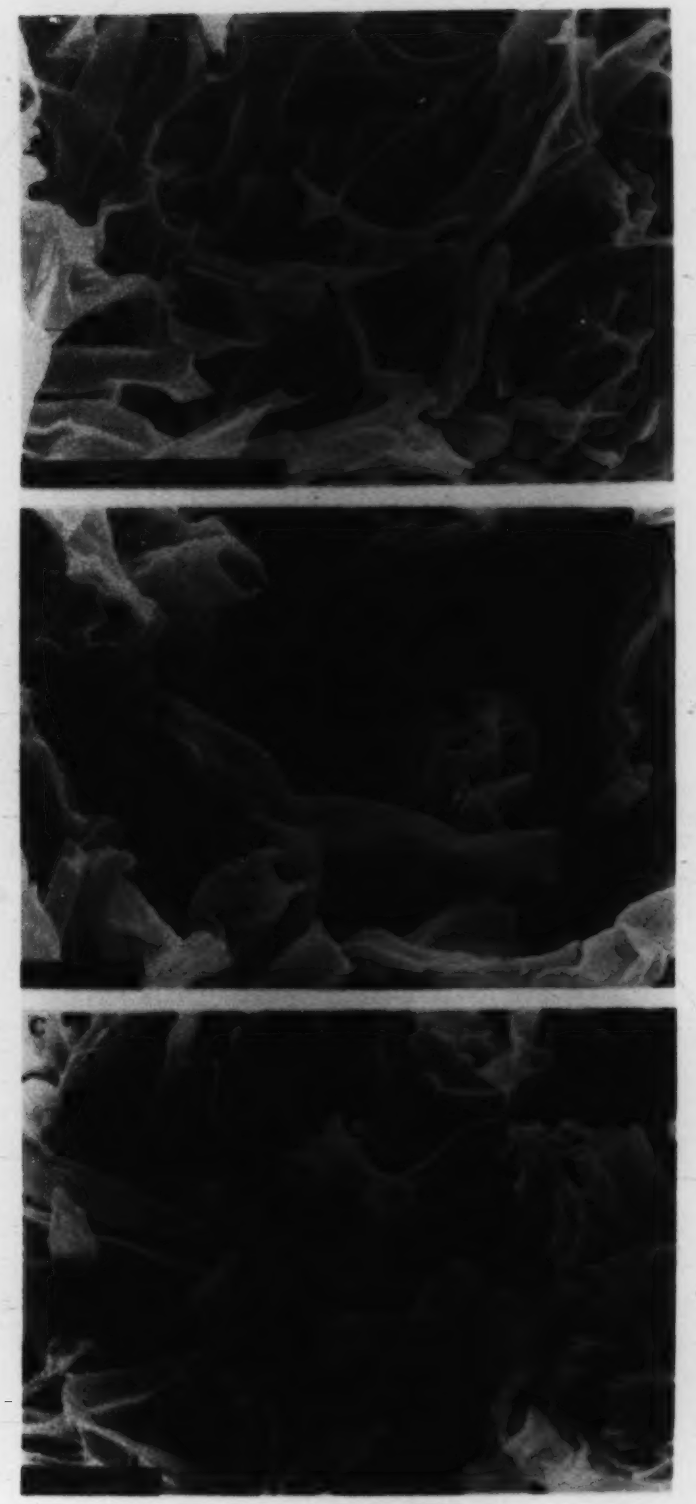

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Plate 9 Scanning electron micrographs of gill surface of dried *P. sajor-caju* mushrooms.
A- Air-dried. B- Freeze-dried. C- Vacuum-dried.

Plate 10 Scanning electron micrographs of hyphae from caps of rehydrated *P. sajor-caju* mushrooms.
A- Air-dried. B- Freeze-dried. C- Vacuum-dried.

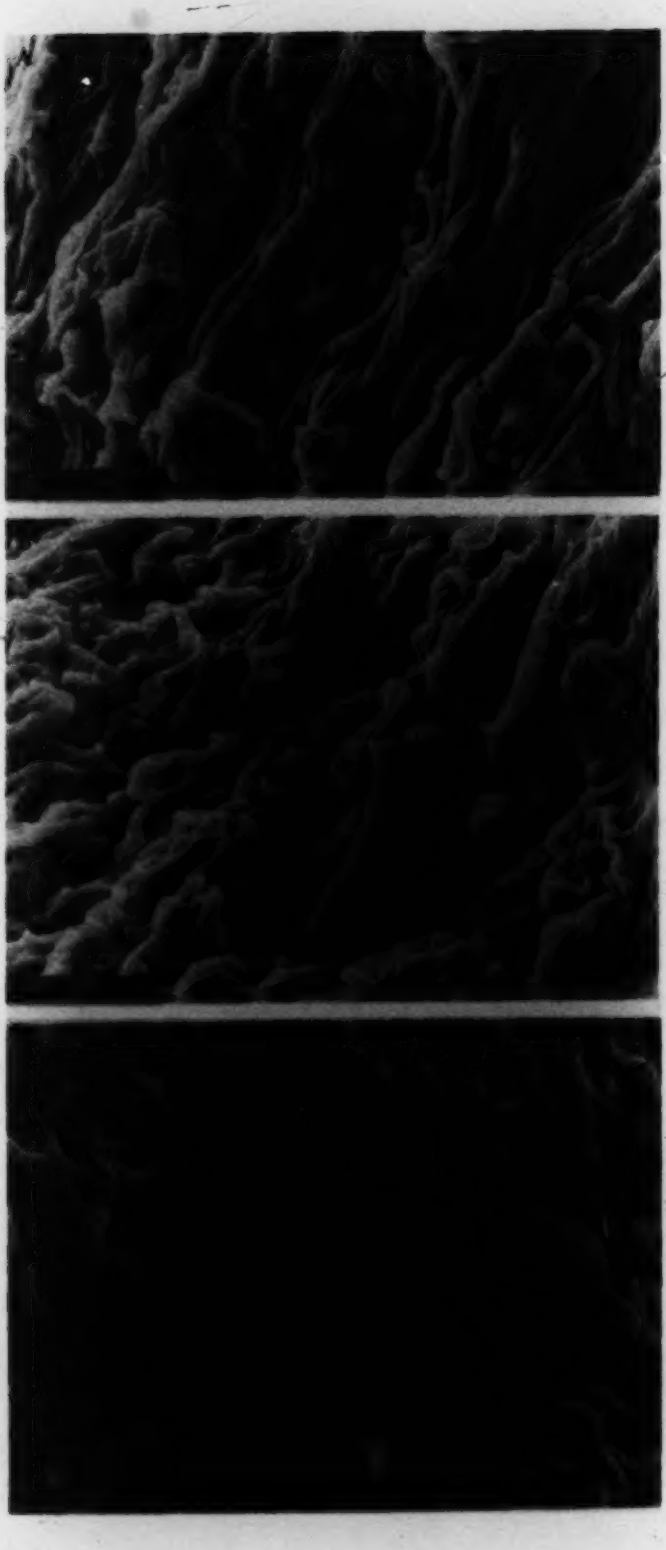

Plate 11 Scanning electron micrographs of gill surface from caps of rehydrated *P. sajor-caju* mushrooms.
A- Air-dried. B- Freeze-dried. C- Vacuum-dried

to the deeper layers while water migrates to the surface where it is evaporated. Additional removal of water at the surface leads to further deformation as the structural elements are crumpled or folded to occupy a shrinking volume.

During freeze-drying, the original dimensions of the product are maintained first by freezing. The ice is then sublimed, usually under a high vacuum. Since there is no aqueous phase, there is no migration of water to the surface but instead a receeding interface of frozen and dry layer. The effects of shrinkage and concentration are thereby prevented [Karel, 1975] and the resulting porous product is not shrunken. At the microstructural level, this was observed as hyphae which had retained to some degree their tubular structure. The basidia, however, were more damaged and some showed perforations in their wall (Plate 9b).

During freezing mechanical injury can result from the growth of ice crystals which occupy a larger volume than water. According to Kuprianoff [1962], if the ice crystals are large enough, physical rupture can occur. Slow freezing has been reported to favour the formation of large extracellular ice crystals while rapid freezing promotes the formation of small intracellular ice crystals which are less likely to cause structural damage [Fennema, 1975]. In this experiment, the mushrooms were frozen by air-blast freezing at 30°C. At this rate of freezing, there could have been the formation of ice crystals large enough to cause structural damage. To assess the extent of the damage, however, further observations at the ultrastructural level by electron transmission microscopy need to be carried out. Bello *et al.* [1984] showed that even with rapid freezing, damage to the nuclei and mitochondria of skeletal fish muscle could be observed. Smaller ice crystals may not be necessarily desirable for the freeze-drying process, since they result into pores of smaller diameter which offer a greater resistance to heat and mass transfer during drying [Ciobanu *et al.*, 1976].

3.2.3.2 Temperature profile during Vacuum Drying

Figure 3 shows the location of the temperature probes in the mushroom cap. There was an initial rise in the temperature of the tissue but after five minutes, the temperature dropped steadily reaching its lowest point after fifteen minutes (Figure 4) when the pressure within the drier was one Torr (Figure 5).

As the pressure within the drier was being reduced, water evaporated rapidly from the mushroom. Heat was lost from the tissue as latent heat of vaporisation but at the same time there was a heat gain from the heating plate and the surroundings. While the other parts of the mushroom cap remained above 0°C at the time intervals recorded, the temperature dropped to -8.2°C in the centre of the cap. Whether that area became supercooled or frozen has not been determined.

The porosity of the vacuum-dried mushroom indicates the possibility of a similar mode of mass transfer in the tissue as that occurring during freeze-drying. Instead of a migration of water which would pull the tissues together, there could have been a receeding interface of moist and dry layer which resulted in a porous structure. Thus, the vacuum-dried hyphae had retained their tubular shape to a similar degree as that observed for the freeze-dried hyphae. The gill surface of the vacuum-dried sample, on the other hand, was not as damaged as the freeze-dried sample, probably due to the omission of the freezing process. The kinetics of heat and mass transfer in the mushroom tissue would have to be investigated further before an explanation can be given for the similar structure of the freeze-dried and vacuum-dried samples.

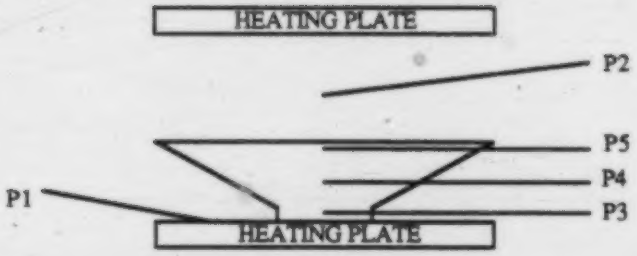
HEATING PLATE
P2
P5
P4
P1
P3
HEATING PLATE

Figure 3. Schematic diagram showing location of temperature probes in a mushroom cap during freeze-drying.

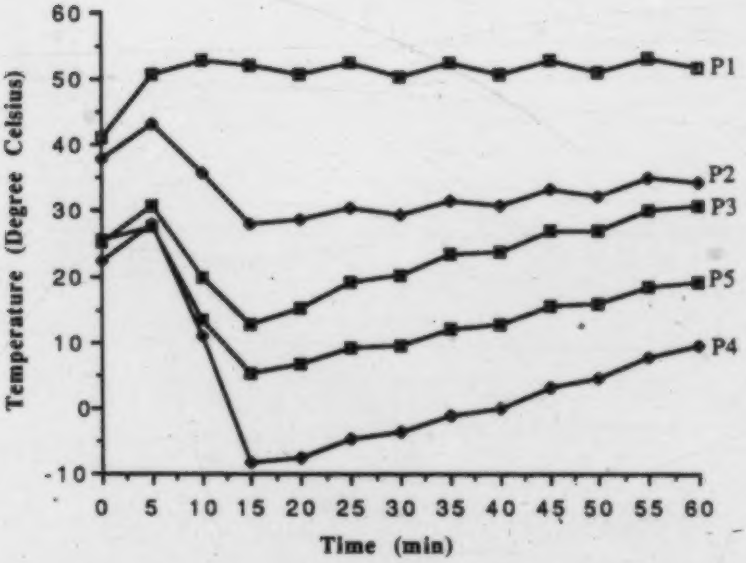

Figure 4. Temperature changes during vacuum-drying of mushrooms

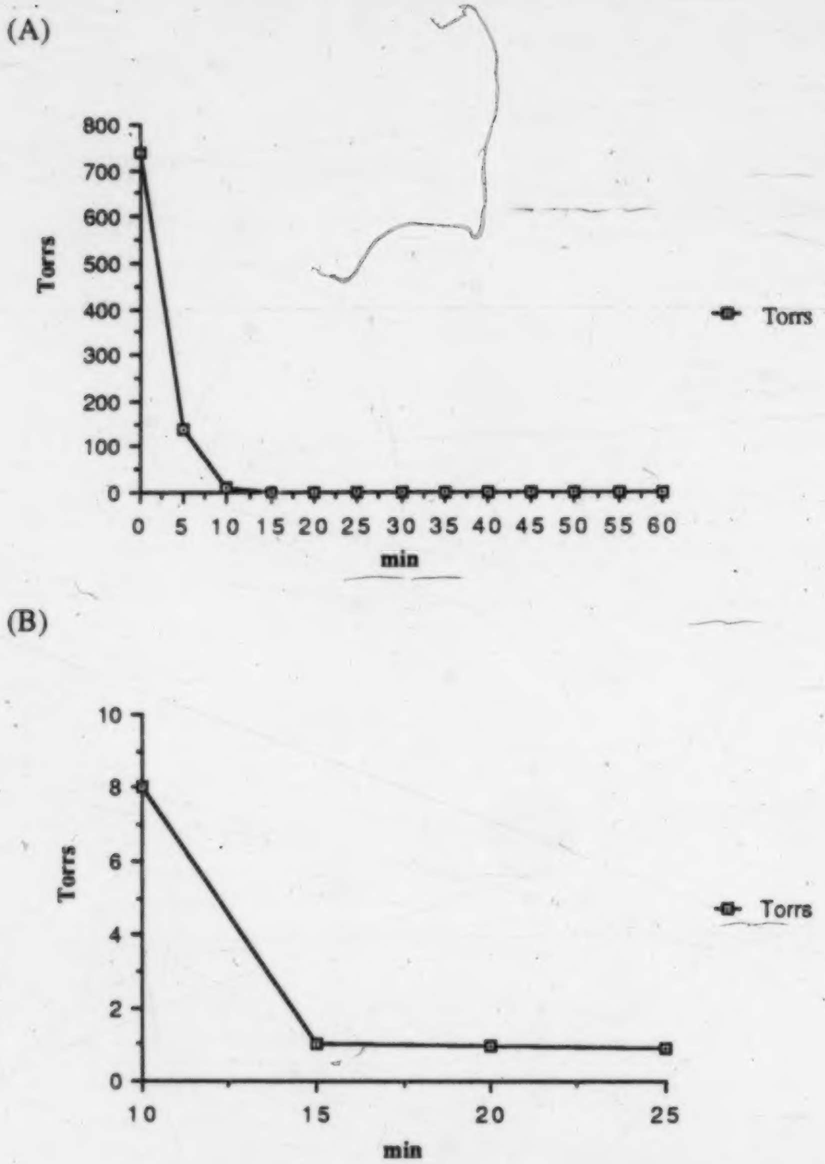

Figure 5. Changes in pressure in the drier during vacuum-drying (A) the first hour, (B) the first twenty-five minutes.

3.2.3.3 Rehydration Properties

The time taken to rehydrate the air-dried *P. sajor-caju* mushrooms was about 40 minutes at 20°C and 5 minutes at 90°C. These values are in agreement with those obtained by Gormley and O'Riordain [1975] for air-dried *P. ostreatus* mushrooms. The vacuum-dried and freeze-dried products were similar in their ability to rehydrate rapidly within 2 minutes at 20°C and in less than 1 minute at 90°C (Table 16). This indicated that the vacuum-dried product had a porous structure similar to that of the freeze-dried product.

Table 16 Rehydration time (minutes) of air-dried, vacuum-dried and freeze-dried *P. sajor-caju* mushrooms

Samples	Moisture (%)*	20°C**	90°**C
Air-dried	7.4 (±0.1)	40 min	5 min
Vacuum-dried	7.6 (±0.1)	2 min	0.75 min
Freeze-dried	7.6 (±0.3)	1.5 min	0.5 min

*Mean values ±S.E (n=9)

**Mean values (n=9)

There was no difference between the vacuum- and freeze-dried products in their rehydration capacity while that of the air-dried samples was significantly lower (Table 17). At the microstructural level, this was reflected by the more wrinkled appearance of the gill surface (Plate 11) and the hyphae (Plate 10) of the rehydrated air-dried samples. The lower rehydration capacity of the air-dried products can be attributed to the irreversible damages on the structure. An impermeable layer resulting from "case hardening" below the surface of the mushroom cap would also hinder water movement back into the tissue. The porous structure of the vacuum-dried product allowed water to be retained to a rehydration capacity similar to that of the freeze-dried product. The lower rehydration capacity values at 90°C are probably due to the loss of water evaporated from the hot materials while they were being weighed. The trend, however, was similar at 20°C, the rehydration capacity of the vacuum- and freeze-dried products being similar while that of the air-dried samples was lower.

Table 17 Moisture content and rehydration capacity of air-dried, vacuum-dried, and freeze-dried *P. sajor-caju* mushrooms (g water/g dried product)

Samples	Batches	20°C	90°C
Air-dried	1	6.36 (±0.14)*	5.50 (±0.11)*
	2	6.46 (±0.03)	5.36 (±0.09)
	3	6.33 (±0.04)	5.42 (±0.00)
	Mean (±S.E)**	6.39 (±0.06)^a	5.44 (±0.05)^c
Vacuum-dried	1	7.01 (±0.04)	6.44 (±0.08)
	2	7.39 (±0.10)	6.45 (±0.08)
	3	7.10 (±0.05)	6.23 (±0.02)
	Mean (±S.E)	7.16 (±0.05)^b	6.40 (±0.04)^d
Freeze-dried	1	7.08 (±0.01)	6.53 (±0.15)
	2	7.23 (±0.01)	6.41 (±0.09)
	3	7.32 (±0.43)	6.52 (±0.08)
	Mean (±S.E)	7.21 (±0.05)^b	6.49 (±0.06)^d

*(n=3)

** (n=9)

a,b,c,d - means with different superscripts are significantly different ($P \leq 0.05$)

3.2.3.4 Texture measurements

Results of texture measurements of rehydrated samples are given in Table 18. Analysis of Variance showed that there was no significant difference between the batches but that the treatments differed significantly. The force required to shear through the rehydrated freeze-dried and vacuum-dried samples was not significantly different showing that they were similar in texture. The air-dried samples were tougher as reflected by the significantly higher shear value. This toughness can be attributed to the irreversible changes produced by the air-drying. According to Van Arsdel [1973], the previously crumpled structures are unable to revert back to their original configurations; also solutes are lost into the rehydration water instead of remaining into the tissue to contribute to turgor recovery. These texture measurements, however, only give an indication of the relative tenderness or toughness of the rehydrated samples. Which texure is more desirable from the consumer's point of view can only be evaluated from sensory analysis.

Table 18 Force (kg) required to shear through the rehydrated *P. sajor-caju* mushrooms (using the Ottawa Texture Measuring System)

Samples	Batches	Maximum force (Peak height) kg/25 g sample
Air-dried	1	72.05 (±1.77)*
	2	73.20 (±3.09)
	3	75.37 (±2.63)
	Mean (±S.E)**	73.54 (±1.43)^a
Vacuum-dried	1	54.10 (±2.74)
	2	50.83 (±1.51)
	3	49.72 (±2.15)
	Mean (±S.E)	51.55 (±1.27)^b
Freeze-dried	1	53.45 (±2.90)
	2	45.95 (±1.65)
	3	50.92 (±1.03)
	Mean (±S.E)	50.11 (±5.64)^b

*(n=3)

**(n=9)

a,b - means with different superscripts are significantly different ($P \leq 0.05$)

3.2.3.5 Sensory evaluation

Panelists of the sensory tests indicated no significant difference in the appearance of the fresh and dried samples after they had been cooked. As expected the fresh mushrooms scored better in all the other attributes. The freeze-dried and vacuum-dried mushrooms were similar with respect to appearance, texture, flavour and overall acceptability (Table 19). The flavour of the vacuum-dried mushrooms was considered better compared to the air-dried products while no significant difference was found between the freeze-dried and air-dried samples. This indicated that the vacuum-dried product was slightly better in flavour than the freeze-dried product but the difference was not large enough to be statistically significant in this evaluation. There could have been better retention of the flavour components in the vacuum-dried products. Alternatively the level of these compounds in both the vacuum-dried and freeze-dried samples might have been similar but there was greater leakage through the damaged walls of the latter during rehydration. The greater damage observed on the gill surface of the freeze-dried product would support the latter explanation. An objective method such as the analysis of particular flavour components would determine whether any real difference existed between the two samples.

The overall score for the texture of the three types of the dried mushrooms after cooking was not, on the whole, significantly different. Many panelists observed that the air-dried mushrooms were tough and rubbery and gave that sample a low score while some actually preferred the texture of the air-dried samples over the softer texture of the other two samples. Also the rehydrated air-dried products softened to a certain extent after they were cooked. Once rehydrated, the vacuum-dried and freeze-dried samples became darker in colour due to enzymic browning. The overall acceptability of the dried mushrooms was determined by the flavour retention of the samples.

Table 19 Summary of results (mean scores, n=26) from the sensory evaluation of fresh and dried caps of *P. sajor-caju* mushrooms after rehydration and cooking

	Fresh	Vacuum-dried	Freeze-dried	Air-dried
Appearance	5.90	5.90	5.96	6.23
Flavour	7.06	6.10	5.81	5.33
Texture	6.85	5.13	5.33	5.12
Overall acceptability	6.85	6.00	5.75	5.35

Mean scores underscored by the same line are not significantly different at 5% level

3.2.4 CONCLUSION

The freeze-dried and high vacuum dried mushrooms were similar in their appearance, rehydration charateristics, texture and sensory attributes. At the microstructural level, the gill surface of the freeze-dried product was more damaged than that of the high vacuum dried sample while there was not much difference in the hyphae. The air-dried products were less appealing in appearance than the freeze- and vacuum-dried mushrooms.

CHAPTER 4

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

4.1 CONCLUSIONS

4.1.1 Cultivation experiments

This study showed that while Aspen shavings per se supported the growth of *Pleurotus*, they did not produce a significant yield of mushrooms. Soaking the shavings in liquid cottage cheese whey increased the yield by more than two-fold (from 3.68% to 8.6%) but would still be inadequate for commercial production. Furthermore, the whey-soaked shavings were particularly susceptible to infection by *Trichoderma*.

The normal strain (P_2) and the sporeless stain (Sp_2) did not produce a significantly different yield with an average of 4.63% and 5.3% (wet weight substrate) from two flushes respectively. However, the Sp_2 fruitbody had a lower cap to stem ratio compared to P_2, which makes it less acceptable to the consumer since the stem was more than two and a half times tougher in texture than the cap. Scanning electron microscopy showed that the basidia of the Sp_2 fruitbody did not bear any spore, confirming its sporelessness. Its potential for cultivation, however, appears to be limited by its morphological characteristics.

Canola meal was found to be a suitable source of supplementation to increase the yield of *P. sajor-caju* mushrooms from Aspen shavings. Its effects on yield were similar to those obtained from using low fat soya flour as a supplement. The best level of canola meal supplementation found in this study was 5%. At a higher level of supplementation, mycelial growth was inhibited. The yield of the two flushes on canola meal supplemented shavings were 16.59%, and 22.14% for a C:N ratio of 70:1, and 47:1 respectively. These cultivation experiments helped to demonstrate the relative simplicity of the cultivation method for *Pleurotus*. Since Aspen wood is an abundant natural resource in Alberta and canola meal is a relative cheap by-product of the local industries, the use of Aspen shavings with canola meal supplementation for the cultivation of *P. sajor-caju* mushrooms may have good potential for commercial application.

4.1.2 Drying experiments

The processing experiments on *Pleurotus sajor-caju* showed that the three drying methods investigated gave acceptable products once they were rehydrated and cooked. The appearance of the vacuum- and freeze-dried mushrooms, however, was much better than the air-dried products which were darker and more shrunken. The vacuum- and freeze-dried mushrooms were very similar in shape and colour to the fresh produce, and would probably appeal better to the consumer. However, their processing costs would make them more expensive than the air-dried products and they would also need special packaging to protect their delicate structure.

Freeze-drying is regarded as the best drying method for the preservation of appearance and flavour of foods. This investigation showed that the high vacuum drying method can give a product similar to that of freeze-drying, with the advantage of bypassing the freezing process. Freezing is believed to cause microstructural damage and in this study, the scanning electron micrographs revealed less damage to the gill surface of the vacuum-dried products compared to those of the freeze-dried mushrooms. The basidia on the gill surface of the freeze-dried sampes looked more collapsed and showed some perforations. Not much difference, however, could be observed in the hyphae of the vacuum- and freeze-dried mushrooms. The hyphae had maintained their tubular structure to some extent in contrast to those of the air-dried samples which were flattened and compact. Scanning electron micrographs of the rehydrated samples reflected the observations made on the rehydration capacity of the dried samples. The gill surface of the rehydrated freeze- and vacuum-dried samples was smooth compared to the more folded appearance of the air-dried samples which had a lower rehydration capacity. The vacuum-dried products rehydrated just as fast as the freeze-dried ones (within 2 minutes), indicating the similarity in the porosity of both structures. The rehydrated air-dried samples were much tougher in texture as demonstrated by a shear value of more than one and a half time those of the vacuum- and freeze-dried samples.

Sensory evaluation of the cooked mushrooms indicated no significant difference in the appearance, flavour, texture, and overall acceptability between the vacuum-dried and freeze-dried products. High-vacuum drying could therefore be a method of potential importance for drying mushrooms. Although vacuum-drying and freeze-drying gave superior products to those of air-drying, it should be pointed out that air-drying was a suitable method for *P. sajor-caju* considering the relative processing costs. The air-dried samples rehydrated in 40 minutes and gave acceptable cooked products.

The importance of these drying experiments lies particularly in the application to high value mushrooms which cannot be satisfactorily air-dried. One such example is the Chanterelle (*Cantharellus cibarius*). The air-dried products are considerably darkened and shrunken, and they take more than eight hours to rehydrate. Freeze-dried Chanterelles rehydrate within a few minutes but are very expensive (Holzbauer, personal communication). If high vacuum-drying can give quality dried Chanterelles at lower costs, this method could be considered as an alternative to freeze-drying Chanterelles. The production costs of high vacuum drying over freeze-drying will have to be assessed in order to determine whether there is any significant economic advantage in omitting the freezing stage.

Preliminary tests on the Chanterelles showed that the vacuum-dried products were indeed similar to the freeze-dried ones (Plate 12).

VACUUM-DRIED
FREEZE-DRIED
AIR-DRIED

Plate 12 Fresh and dried Chanterelles (*Cantharellus cibarius*)

4.2 Suggestions for future work

The similarity of the high vacuum dried mushrooms to the freeze-dried product is particularly interesting. It is possible that there is a similar mode of mass transfer in the tissue to that occurring during freeze-drying to account for the porous structure. However, this would need to be investigated further. The temperature profile of the centre of the mushroom recorded a drop in temperature to below -8°C but whether that area became supercooled or frozen is an interesting question that needs to be answered. The vacuum-drying experiments should be repeated at different pressures, temperatures, and time periods to evaluate the effects on the fruitbodies. The maintenance of a high vacuum during drying is expensive. Using a lower vacuum might give products intermediate in rehydrating properties between the air-dried and freeze-dried samples. The microstructural observations were limited to the scanning electron micrographs. Further observations at the ultrastructural level using the transmission electron microscopy should give a better picture of the effects of the three drying methods.

The panelists used in the sensory evaluation tests were untrained. A smaller group of trained panelists might have been able to distinguish better the differences in the attributes of the various samples and ideally, the samples should be tested after different periods of time to assess any storage effect. This involves a long term planning and would be a project in itself. The sensory tests carried out in this work, however, helped to indicate the general acceptability of the products. To find out whether there is any real significant difference in the flavour between the vacuum-, freeze-, and air-dried samples, chemical analysis of the flavour components would need to be carried out. However, the flavour chemistry of mushrooms is already a complex subject and little research has been done on the flavour components of the *Pleurotus* mushrooms.

Only the caps were used here for drying as they are the parts that are preferred by the consumers. The stems, however, could still be dried and used in several products, e.g. an 'Oyster mushroom soup'. The acceptability of different soup formulations would need to be tested by sensory evaluation. The presence of antitumour substances in various mushrooms has been reviewed in Chapter 2. One area of potential importance to the pharmaceutical industry is to investigate the concentration of these substances in the stems, their extraction rates and their eventual use as pharmacological compounds. Mori [1987] has demonstrated the inhibiting, and sometimes complete regression effects of *Pleurotus* mushroom extracts on tumours transplanted into mice. These medical effects should be investigated further.

Mushroom cultivation has often been described as an "art and a science", and for *Pleurotus* cultivation this is true in many respects. Many of the cultivation practices are based on previous observations. Although these cultivation steps are founded on some general concepts, more thorough explanations are still lacking. The biological nature of the cultivation process is such that one has to deal with the variations between species and strains of the same species growing on a wide variety of substrates under different environmental conditions. This means that the results of a successful operation cannot be necessarily extrapolated to other situations. In this study, particular strains of *P. ostreatus* and *P. sajor-caju* were used. Future cultivation studies could explore the growth of other strains and species. Some sporeless varieties of *Pleurotus* are now available from spawn companies.

In this work, substrate preparation was kept as simple as possible. Apart from the organic source of supplementation, no other compound was added and in most cases, the substrate was sterilised. For a large scale operation, dealing with sterilised substrate may not be practical. Fermentation as a method of heat treatment for supplemented Aspen shavings as a substrate should be explored. The nutritional requirements of *Pleurotus* mushrooms are not well understood and much more research needs to be done before substrate supplementation can be optimised.

Very few postharvest studies have been reported so far on Oyster mushrooms. The information available for the *Agaricus* mushrooms is not necessarily applicable to *Pleurotus* mushrooms since they differ not only morphologically but chemically as well. At the moment *Pleurotus* mushrooms are sold mostly fresh. Research should therefore be directed in finding ways of extending the shelf-life of the fresh produce. Vacuum cooling and irradiation are two methods worth exploring. Freezing and canning should be studied further to assess their effects on the quality on the fruitbodies. The acceptability of the processed mushrooms to the consumer should be evaluated. Proper packaging is not only important in maintaining the quality of the mushrooms on the shelf but should be so designed as to appeal to the consumers as well.

The "exotic" mushroom industry which is attempting to explore the cultivation of mushroom species other than the button mushroom has only recently been established in North America. There is consequently a tremendous amount of research that needs to be carried out in both the cultivation and the processing areas.

CHAPTER 5

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CHAPTER 6

APPENDICES

6.1 Appendix A

This appendix contains the results of the analysis of variance tests on the data from the rehydration experiments, the texture analysis, and the sensory evaluation tests.

Table A1. Anova table for rehydration capacity at 20°C

Source of variation	d.f.	Sum of Squares	Mean Square	F ratio
Batches	2	0.19672	0.09836	3.5078
Treatments	2	3.85956	1.92978	68.8223*
Batches x Treatments	4	0.15635	0.03909	1.3941
Error	18	0.50467	0.02804	
Total	26	4.7173		

*significant difference at 1% level

Table A2. Anova table for rehydration capacity at 90°C

Source of variation	d.f	Sum of Squares	Mean Square	F ratio
Batches	2	0.06794	0.03397	1.4160
Treatments	2	5.89116	2.94558	122.7837*
Batches x Treatments	4	0.10055	0.02514	1.0479
Error	18	0.43173	0.02399	
Total	26	6.49139		

*significant difference at 1% level

Table A3. Anova table for texture analysis of the Phoenix Oyster mushroom

Source of variation	d.f.	Sum of Squares	Mean Square	F ratio
Batches	2	94.42704	47.21352	1.53279
Treatments	2	6208.31259	3104.1563	100.77641*
Batches x Treatments	4	176.54185	44.13546	1.43286
Error	45	1386.10833	30.80241	
Total	5	3865.38981*		

*Significant difference at 1% level

Table A4. Anova table for appearance score of the Phoenix Oyster mushroom

Source of variation	d.f.	Sum of Squares	Mean Square	F ratio
Treatments	3	3.81	1.27	0.88
Replicates	1	0.48	0.48	
Panelists	25	122.00	4.88	3.39*
Treatments x Panelists	75	157.19	2.10	1.46
Error	103	148.52	1.44	
Total	207	432.00		

*Significant difference at 1% level

Table A5. Anova table for flavour score of the Phoenix Oyster mushroom

Source of variation	d.f.	Sum of Squares	Mean Square	F ratio
Treatments	3	83.05	27.68	11.21*
Replicates	1	0.12	0.12	
Panelists	25	79.29	3.17	1.28
Treatments x Panelists	75	193.07	2.57	1.04
Error	103	254.38	2.47	
Total	207	609.92		

*Significant difference at 1% level

Table A6. Anova table for texture scores of the Phoenix Oyster mushroom

Source of variation	d.f	Sum of Squares	Mean Square	F ratio
Treatments	3	3.81	1.27	0.88
Replicates	1	0.48	0.48	
Panelists	25	122.00	4.88	3.39*
Treatments x Panelists	75	157.19	2.10	1.46
Error	103	148.52	1.44	
Total	207	432.00		

*Significant difference at 1% level

Table A7. Anova table for overall acceptability scores of the Phoenix Oyster mushroom

Source of variation	d.f.	Sum of Squares	Mean Square	F ratio
Treatments	3	62.67	20.89	14.11*
Replicates	1	0.39	0.39	
Panelists	25	100.08	4.00	2.70*
Treatments x Panelists	75	149.71	2.00	1.35
Error	103	152.11	1.48	
Total	207	464.96		

*Significant difference at 1% level

6.2 Appendix B

This appendix contains the taste panel score sheet which was used for the sensory evaluation, the scores from the panelists, and the results of the Tukey's test for each of the sensory attributes tested.

TASTE PANEL SCORE SHEET

Name---------------------- Date---------

Evaluate each sample by checking how much you like, or dislike it. Use the scoring guide listed below for each attribute of each sample, and include any comments you may have. Please taste each sample in the order presented on this score sheet.

1=dislike extremely
2=dislike very much
3=dislike moderately
4=dislike slightly
5=neither dislike nor like
6=like slightly
7=like moderately
8=like very much
9=like extremely

Sample Number

Attributes	#	#	#	#
Appearance				
Texture				
Flavour				
Overall acceptablility				

Comments:

Table B1. Scores for the sensory attributes of the fresh and air-dried mushrooms

Sample	Attributes	Panelists																									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Fresh	Appearance	5	5	4	6	6	4	7	6	5	5	6	6	8	5	8	7	4	7	3	6	5	6	4	6	8	7
		7	7	5	6	5	6	8	5	5	6	6	7	6	8	7	5	7	5	4	5	5	5	6	8	8	6
	Texture	9	8	6	7	8	5	5	7	6	6	7	8	8	8	7	7	8	4	6	7	5	9	2	7	8	8
		8	7	9	8	5	8	7	7	7	7	6	8	8	8	8	7	7	3	6	6	6	7	6	7	7	7
	Flavour	9	8	7	8	9	7	7	8	7	6	8	7	7	8	6	8	8	6	9	8	6	8	5	8	4	8
		8	7	8	8	4	4	5	7	7	7	7	8	6	7	8	8	8	6	8	7	6	7	7	4	7	8
	Overall Acceptability	8	8	7	7	7	5	7	7	6	6	7	7	8	8	7	7	7	6	8	8	4	7	4	7	7	8
		8	7	8	8	4	6	6	7	7	7	6	8	7	8	8	7	8	5	7	7	6	6	6	7	7	7
Air-dried	Appearance	9	5	8	8	3	5	6	7	5	5	8	5	7	6	5	7	7	6	7	6	4	3	7	7	8	7
		6	5	6	7	6	7	6	7	4	6	7	7	7	8	8	7	7	6	4	6	6	3	6	5	8	8
	Texture	6	2	4	3	6	4	6	7	5	3	8	6	7	8	3	5	5	5	4	6	4	6	6	6	8	4
		4	2	4	2	4	7	7	4	5	7	6	6	7	7	6	7	5	2	2	4	4	3	6	4	7	7
	Flavour	6	4	7	3	4	4	6	8	5	4	8	8	7	4	5	2	5	7	2	7	4	7	7	7	9	4
		4	3	8	3	5	8	7	6	5	6	5	6	5	6	6	4	6	4	2	5	5	4	6	6	2	6
	Overall Acceptability	6	3	7	3	5	4	6	8	5	4	8	7	7	5	3	4	5	6	3	6	5	5	7	7	9	4
		5	2	7	3	5	7	7	6	5	6	6	6	6	6	6	4	6	4	3	5	5	4	6	5	5	6

Table B1. (continued) Scores for the sensory attributes of the vacuum-dried and freeze-dried mushrooms

Sample	Attributes	Panelists																									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Vacuum-dried	Appearance	1	5	3	4	4	6	6	5	6	6	4	6	7	5	8	7	7	6	6	7	5	8	5	6	8	7
		4	9	5	8	6	4	6	6	6	6	5	6	5	7	8	5	7	6	6	6	6	4	6	7	8	7
	Texture	6	3	5	6	1	6	6	7	4	5	5	8	7	3	8	6	5	6	8	6	4	8	1	4	6	5
		2	9	3	4	5	2	5	7	4	6	6	7	5	4	8	5	3	5	6	5	3	3	5	5	5	6
	Flavour	8	4	9	6	3	7	4	3	4	6	6	8	6	7	8	6	7	8	7	6	5	6	3	5	4	5
		3	9	6	6	6	5	7	8	3	8	7	6	8	7	8	5	3	8	9	6	6	7	7	5	8	5
	Overall Acceptability	7	3	8	5	4	6	5	5	5	6	5	8	7	5	8	6	7	7	7	6	5	8	3	5	6	5
		3	9	6	6	6	5	6	8	4	7	6	7	7	7	8	5	4	7	8	6	4	5	6	6	8	6
Freeze-dried	Appearance	3	5	6	7	7	5	7	7	8	6	8	6	6	5	8	3	7	4	7	4	5	9	6	5	9	8
		3	5	5	6	4	2	7	7	4	7	8	8	5	7	8	5	5	5	6	7	6	7	4	5	8	5
	Texture	6	5	6	4	3	5	6	4	6	5	5	7	5	6	8	6	3	3	8	7	5	6	3	4	8	6
		2	4	7	3	5	6	5	6	5	7	7	7	5	4	7	5	6	4	4	6	4	4	5	6	8	5
	Flavour	7	8	8	4	3	6	6	5	7	6	5	6	5	7	7	3	2	3	6	6	7	6	6	6	9	5
		3	5	9	6	6	9	5	5	5	8	7	8	5	7	7	5	5	6	5	8	5	6	4	4	6	4
	Overall Acceptability	6	7	8	4	3	5	6	5	7	6	6	6	5	7	7	3	2	3	7	6	6	8	4	5	9	5
		4	5	8	5	6	8	5	7	5	8	7	8	5	7	7	5	5	5	4	8	5	6	5	4	7	4

Table B2. Comparison of means using Tukey's test for the appearance of fresh and dried samples of the Phoenix Oyster mushroom

Sequence of means

4	3	2	1	
	5.90	5.95	5.96	6.23

Table of differences

	4	3	2	1
1	0.33	o.28	0.27	0.00
2	0.06	0.01	0.00	
3	0.05	0.00		
4	0.00			

Table of test values

	4	3	2	1
1	0.62	0.62	0.62	
2	0.62	0.62		
3	0.62			

Air-dried	Freeze-dried	Vacuum-dried	Fresh
4	3	2	1

Samples underlined by the same line showed no significant difference at the 5% level

Table B3. Comparison of means using Tukey's test for the flavour of fresh and dried samples of the Phoenix Oyster mushroom

Sequence of means

4	3	2	1	
	5.33	5.81	6.10	7.06

Table of differences

	4	3	2	1
1	1.73	1.25	0.96	0.00
2	0.77	0.29	0.00	
3	0.48	0.00		
4	0.00			

Table of test values

	4	3	2	1
1	0.62	0.62	0.62	
2	0.62	0.62		
3	0.62			

Air-dried	Freeze-dried	Vacuum-dried	Fresh
4	3	2	1

Samples underlined by the same line showed no significant difference at the 5% level

Table B4. Comparison of means using Tukey's test for the texture of fresh and dried samples of the Phoenix Oyster mushroom

Sequence of means

4	3	2	1
5.12	5.13	5.33	6.85

Table of differences

4	3	2	1	
1	1.73	1.72	1.52	0.00
2	0.21	0.20	0.00	
3	0.10	0.00		
4	0.00			

Table of test values

	4	3	2	1
1	0.62	0.62	0.62	
2	0.62	0.62		
3	0.62			

Air-dried	Freeze-dried	Vacuum-dried	Fresh
4	3	2	1

Samples underlined by the same line showed no significant difference at the 5% level

Table B5. Comparison of means using Tukey's test for the overall acceptability of fresh and dried samples of the Phoenix Oyster mushroom

Sequence of means

4	3	2	1	
	5.35	5.75	6.00	6.85

Table of differences

	4	3	2	1
1	1.50	1.10	0.85	0.00
2	0.65	0.25	0.00	
3	0.40	0.00		
4	0.00			

Table of test values

	4	3	2	1
1	0.62	0.62	0.62	
2	0.62	0.62		
3	0.62			

Air-dried	Freeze-dried	Vacuum-dried	Fresh
4	3	2	1
			———
	———	———	
———	———		

Samples underlined by the same line showed no significant difference at the 5% level